Marine Ornamental Shrimp

Biology, Aquaculture and Conservation

Marine Ornamental Shrimp

Biology, Aquaculture and Conservation

Ricardo Calado, PhD

CCMAR, University of the Algarve, Portugal
CESAM, University of Aveiro, Portugal

WILEY-BLACKWELL
A John Wiley & Sons, Ltd., Publication

This edition first published 2008

Blackwell Publishing was acquired by John Wiley & Sons in February 2007.
Blackwell's publishing programme has been merged with Wiley's global
Scientific, Technical, and Medical business to form Wiley-Blackwell.

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Editorial office
9600 Garsington Road, Oxford, OX4 2DQ, United Kingdom
2121 State Avenue, Ames, Iowa 50014-8300, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

Library of Congress Cataloging-in-Publication Data
Calado, Ricardo.
Marine ornamental shrimp : biology, aquaculture, and conservation /
Ricardo Calado. — 1st ed.
p. cm.
Includes bibliographical references and index.
ISBN-13: 978-1-4051-7086-4 (hardback : alk. paper)
ISBN-10: 1-4051-7086-7 (hardback : alk. paper) 1. Ornamental shrimps. 2. Marine aquarium animals. I. Title.

SF458.O76C35 2008
639'.68–dc22

2008002541

A catalogue record for this book is available from the British Library.

Set in 10/13 pt Palatino
by Newgen Imaging Systems (P) Ltd, Chennai, India
Printed in Singapore by C.O.S. Printers Pte Ltd
1 2008

To my son Rodrigo and my wife Tânia

Contents

Foreword

The growing popularity of marine aquarium keeping has allowed us to admire the splendor of organisms that, just a few years ago, could be seen only by those privileged to dive in coral reefs. As in many other hobbies, an associated industry was born, has grown stronger and nowadays represents a multimillion-dollar activity worldwide. Currently, this industry faces global pressure to prove its sustainability. The current state of crisis of coral reefs has put the aquarium industry 'in the line of fire'. In most cases, the collection of organisms from the wild to supply the aquarium industry has been irrationally accused of promoting the destruction of coral reefs. Cyanide fishing, as well as the collection of certain species that may not support current collection pressure, is certainly a serious conservation issue. However, major threats to coral reefs are certainly not promoted by the aquarium trade industry. The devastating and long-lasting effects of dynamite fishing, water quality degradation as a result of anthropogenic pollution, and global warming are certainly more relevant threats. Nevertheless, there is a current effort to advocate and enforce sustainable collection and trade of marine ornamental species. In an attempt to minimize the industry's dependence on the collection of wild specimens, research institutes and private enterprises have started to address the culture of marine ornamental species. This activity presents great potential, and several enterprises have already proved that it can be highly profitable. However, the lack of basic scientific knowledge on many of the species targeted by culture efforts has proved to be a serious bottleneck that impairs commercial-scale culture of some of the most highly demanded species. Despite these constraints, there are now several successful companies, as well as public aquariums, rearing captive produced and/or growing wild-caught post-larval reef fishes, producing giant clams and propagating soft and hard corals. Because of the permanent need to recruit new species and diversify their offerings, enterprises have started to address the culture of marine ornamental shrimps. Supported by preliminary experiments performed by academic researchers, enterprises did not take long to realize that methodologies commonly employed in penaeid shrimp larval culture were not suitable for marine ornamental shrimps. The lack of ecological studies addressing larval development and reproductive biology of these organisms has delayed the progress of marine ornamental shrimp culture. Only after a multidisciplinary

approach was adopted were the initial bottlenecks finally overcome. Aquaculture engineering provided the basis for adapting existing larval rearing systems to the particular needs of marine ornamental shrimp larvae. Larval morphology specialists helped aquaculturists to identify the stages of larval shrimps and thus diagnose unsuitable rearing conditions. Researchers working in shrimp nutrition provided valuable guidelines for maturation, larval and juvenile grow-out diets through the analysis of the biochemical profiles of wild embryos, larvae and juveniles. Creativity also played an important role in the development of suitable sampling devices for ecological studies on marine ornamental shrimps and in the development of imaginative solutions for broodstock management and juvenile grow-out.

The present work brings together the information currently available on the biology, aquaculture and conservation of marine ornamental shrimps. It discusses the major breakthroughs achieved in their culture and identifies current bottlenecks and future improvements to existing methods. The objective of this book is to provide a working tool for all those willing to contribute to the study of marine ornamental shrimps, namely academic researchers, entrepreneurs who wish to address the commercial culture of marine ornamental shrimps, and enthusiastic hobbyists who are always willing to extend their knowledge on the magnificent organisms displayed in their marine aquariums. It is the task of researchers, traders and hobbyists to promote the gradual replacement of wild-collected by captive-cultured organisms in the marine aquarium trade, so as to contribute to its sustainable development. Only by increasing the number of cultured species available for trade will this hobby prosper and continue to show us the beauties of coral reefs.

Ricardo Calado
Lisbon, Portugal

Acknowledgements

The present work was only possible with the help of other fellow researchers, students, marine aquarium industry professionals, hobbyists and friends, who share my passion for marine ornamental species and shrimp biology. I would like to thank: Andrew L. Rhyne from Florida University, for helping me achieve the breakthroughs on ornamental shrimp larval culture in the early 2000s, as I am sure that without our initial enthusiasm, creativity and persistence, marine ornamental shrimp culture would still be facing even greater bottlenecks; Rui Rosa from the Biological Sciences Center — University of Rhode Island, for help on the biochemical studies addressing ornamental shrimps, as well as for being my dive buddy on several campaigns to collect broodstock; Cátia Bartilotti and Antonina dos Santos from Instituto Nacional de Investigação Agrária e das Pescas — Instituto de Investigação das Pescas e do Mar, for all their invaluable help on larval decapod morphology and ecology; Junda Lin from the Department of Biological Sciences — Florida Institute of Technology, for receiving me in his laboratory, making possible the construction of the first research-scale prototype tanks for ornamental shrimps larval culture, and for always being available to critically comment upon my work; Raymond T. Bauer from the Department of Biology — University of Louisiana at Lafayette, for so promptly clarifying my questions concerning the reproductive biology of shrimps, particularly those addressing simultaneous hermaphroditism in the genus *Lysmata*; Sofia Morais from the University of Stirling, for all her help in my earlier research on marine ornamental shrimp culture and biochemical analysis; Luís Narciso from Laboratório Marítimo da Guia — Faculdade de Ciências da Universidade de Lisboa, for supervising my PhD and providing the laboratory conditions to enable me to develop my first studies on marine ornamental shrimps; Ricardo Araújo and Manuel Biscoito from Estação de Biologia Marinha do Funchal, for always being available to receive me in their laboratory and for providing me with the logistics required to collect marine ornamental shrimps in the beautiful island of Madeira; Maria Leonor Nunes from Instituto Nacional de Investigação Agrária e das Pescas — Instituto de Investigação das Pescas e do Mar, for providing the laboratory conditions required in order to perform biochemical analyses on the numerous samples of marine ornamental shrimp embryos, larvae and juveniles; Pierre Chevaldonné from the Centre d'Océanologie de Marseille, Aix-Marseille Université,

Station Marine d'Endoume, for valuable tips on cryptic shrimp collection and for introducing me to the world of shrimp molecular biology; Alberto Reis and Teresa Lopes da Silva, for their collaboration on the biochemical analysis of numerous aspects concerning the life cycle of ornamental shrimps; Cédric d'Udekem d'Acoz, for sharing his vast knowledge on the biology of decapod crustaceans, namely the members of family Hippolytidae; António Marques from Instituto Nacional de Investigação Agrária e das Pescas — Instituto de Investigação das Pescas e do Mar, for his comments on the use of probiotics in aquaculture and for providing photos for this work; Brian Schaff, the owner of the Portuguese enterprise, LusoReef (the first company to raise marine ornamental shrimps on a commercial scale on a regular basis), for providing pictures and valuable information on grow-out and packing techniques; to the Portuguese forum of marine aquarium hobbyists, Reefforum (www.reefforum.net), namely the administrator, Julio Macieira, and members, Diogo Lopes, João Ribeiro, Ricardo Santos and Tiago Garcia, for so promptly providing photos for this work; Luis Magnasco, for his valuable information on *Lysmata amboinensis* culture and for providing photos for this work; Maria Teresa Dinis from Centro de Ciências do Mar da Universidade do Algarve, for supervising my post-doc research on the nutritional aspects of larval, juvenile and adult *Lysmata* and for enabling me to continue to develop my research studies on marine ornamentals; Jorge Dias from Centro de Ciências do Mar da Universidade do Algarve, and Paulo Rema from Centro Interdisciplinar de Investigação Marinha e Ambiental and Universidade de Trás-os-Montes e Alto Douro, for their help on the formulation of inert diets for larval, juvenile and broodstock of marine ornamental shrimps; my former colleagues at Laboratório Marítimo da Guia Lueji Pestana and Gil Penha-Lopes and my students António Vitorino, Gisela Dionísio and Cristovão Nunes, for their dedication and enthusiasm during the experimental trials on marine ornamental shrimps larval culture, reproduction and nutrition; Rui Rocha and Sofia Engrola, for their support and for keeping our enterprise CoralPro running during my absence while writing this work; Kate Nuttall (editorial assistant) and Nigel J. Balmforth (senior publisher) at the Professional Division of Blackwell Publishing, for their help, support and understanding, particularly during the last stages of this work; and finally Tânia, for her unconditional love and support throughout my academic career and for taking care, with the help of Isabel and Joaquim, of our precious newborn baby Rodrigo while I was trying to finish this work.

Chapter 1

Introduction

1.1 The marine ornamentals industry

In recent years, marine aquarium keeping has become a popular activity, with reef tanks being widely considered the most challenging and spectacular of displays. Countries in the Indo-Pacific regions, particularly those in Southeast Asia, as well as those in the Red Sea and the Caribbean, are the main suppliers of marine ornamentals. This highly profitable industry relies almost exclusively on wild specimens, mainly caught in reef areas. This dependence on the collection of wild specimens has been regarded with increasing concern by researchers, local policy makers and responsible traders (Wabnitz *et al.*, 2003). Widespread and growing apprehension over the collection of ornamental species from the wild has forced the industry to progressively abandon unsustainable fishing practices, namely the highly destructive technique of cyanide fishing. The negative impacts directly or indirectly linked to cyanide fishing have resulted in the creation of several laws banning this fishing practice in exporting countries (Pet & Pet-Soede, 1999), with conscientious marine ornamental fish suppliers excluding cyanide-caught specimens from their stock lists. These policies have led to the establishment of quality labels, such as 'cyanide free' or 'net caught' (Rubec *et al.*, 2000), and the Marine Aquarium Council (MAC) has played a key role in global efforts to establish a sustainable industry. Nowadays, the label 'MAC certified' assures hobbyists that the specimens they are acquiring were collected, transported and stocked according to sustainable practices and respecting animal welfare (Bunting *et al.*, 2003). However, the aquarium trade is a complex industry, and tracing a newly acquired organism is far from being a simple task (Figure 1.1).

The culture of heavily traded marine ornamental species has for long been regarded as an alternative to wild specimens capture. However, the lack of knowledge on the reproductive and larval biology of most trade species has resulted in bottlenecks that have impaired commercial-scale culture. Despite such difficulties, the strong belief of some researchers and traders that captive

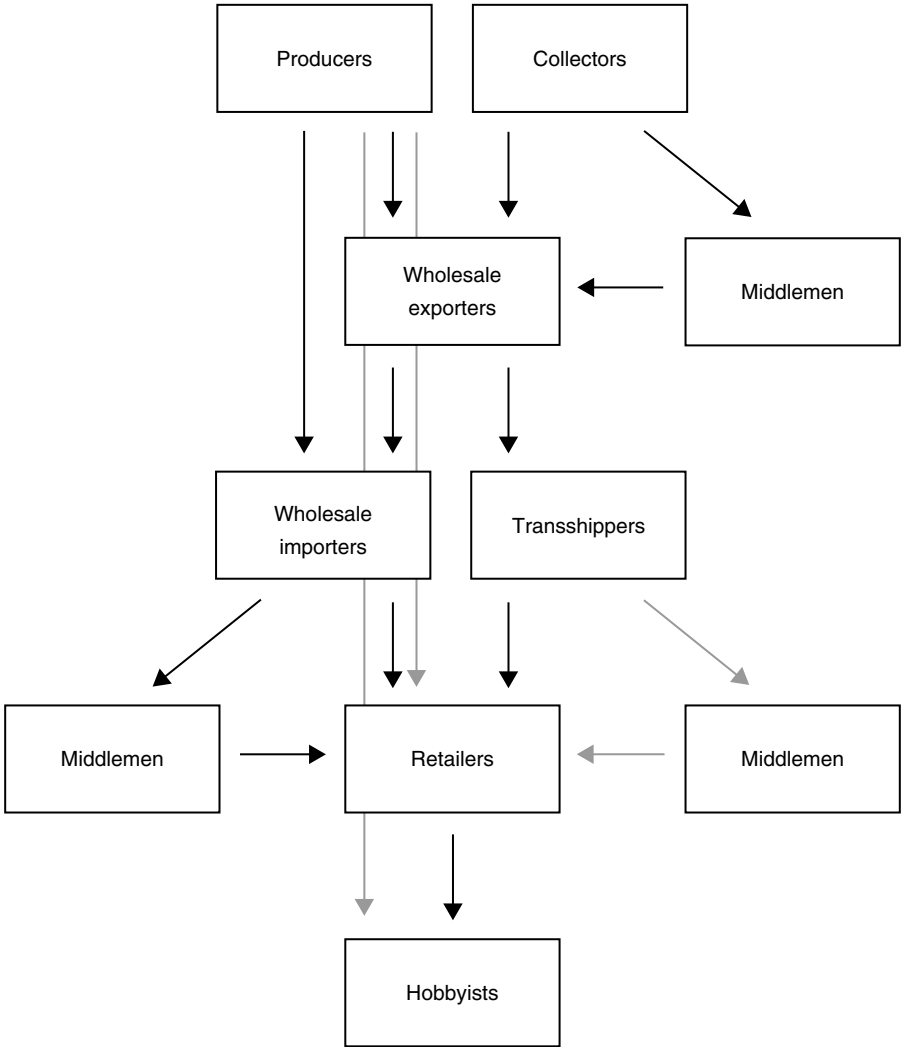


Figure 1.1 Schematic representation of the trade in marine ornamental species (black arrows represent common trade practices and grey arrows less common ones).

culture was more than a potentially profitable commercial venture, and that there was a need for the sustainable development of the industry, has resulted in the development of feasible culture protocols for several species. The number of cultured marine ornamental species is still low (Wabnitz *et al.*, 2003) if compared with the total number of species available in the trade, but has been steadily increasing, with new breakthroughs being achieved with each passing year. Nevertheless, the establishment of innovative approaches concerning the aquaculture of marine ornamental species, namely the collection of wild post-larvae for *ex situ* grow-out, has widened the range of cultured species available in the trade. This new approach needs to be carefully addressed by

researchers, in order to ensure that all target groups of marine ornamentals are able to stand this new fishing effort and that this methodology is indeed a sustainable alternative.

A potential drawback of most current marine ornamentals culture efforts being centred in western countries (namely the USA and the EU countries) is the negative social and economic impacts they may have in exporting countries, particularly those in Southeast Asia and the Central Pacific. It is highly advisable that developed knowhow is shared with exporting countries so as to provide an alternative way of life for impoverished local populations currently surviving through the capture of marine species for the aquarium trade (Tlusty, 2002).

Reef fishes and corals are by far the most heavily collected and traded group of organisms in the marine aquarium industry, representing the major income source in this commercial activity. However, an increasing number of other groups of marine invertebrates are also targeted by professional collectors and enter the marine ornamental species trade. Decapod crustaceans are one of those invertebrate groups experiencing an increase in demand among hobbyists. Of all decapod crustaceans available in the marine aquarium industry, ornamental shrimps have always been the most popular (Debelius, 1984; Shimek, 2004).

1.2 What is a marine ornamental shrimp?

So, what are marine ornamental shrimps? Shrimp species are commonly designated as ornamental if they display one or more of the following features:

(1) they show dazzling coloration;
(2) they have delicate and/or bizarre external morphology;
(3) they display unusual reproductive traits;
(4) they exhibit symbiotic behavior;
(5) they perform fish cleaning;
(6) they control nuisance organisms in the marine aquarium; and
(7) they are 'reef-safe'.

Coloration is probably the most common feature used to recruit shrimp species to the marine aquarium trade. Intense colorations are usually preferred over faded ones, with bright red, orange or yellow shrimps being favoured by the industry. Unusual color patterns (e.g. spots and stripes), iridescent body parts and conspicuous white antennae are also highly regarded by hobbyists. Concerning morphology, ornamental shrimps usually display unique characters that give them a distinctive appearance. Modified claws are highly appealing features, some species of ornamental shrimp displaying enlarged,

paddle-shaped or snapping chelae. Sexually dimorphic morphological features, namely enlarged mouth parts (e.g. the third pair of maxillipeds) and unusually slender or robust bodies may also be used to promote a shrimp species to ornamental status.

The reproductive behavior of several shrimp species has also granted them a place in marine aquariums. Hobbyists usually wish to stock paired specimens in their reef displays. Although the majority of ornamental shrimp species display separate sexes, hermaphroditism is not an unusual feature. Certain species may begin their life as males and later change to females, some being able to retain their male mating ability while in female phase, becoming true euhermaphrodites. While some shrimp species may display 'monogamic' behavior, and are even able to recognize their mating pair after isolation periods, others live in crowds with large dominant primary males trying to keep their harems free from 'female-looking' smaller males. Sometimes aggressive behavior is displayed only towards conspecifics of the same sex, with those from the opposite sex only being tolerated by solitary individuals.

Shrimps displaying symbiotic behavior are also highly prized by marine aquarium hobbyists. The puzzling associations between marine shrimps and numerous other invertebrates, namely cnidarians (e.g. sea anemones, and soft and hard corals), as well as some fish species (e.g. gobies and moray eels) are long-time favorites in coral reef displays. Because of the hobbyist's preference for symbiotic species, it is common that both members of the symbiosis reach higher market values when sold together than separately.

Species exhibiting fish cleaning behavior are also considered a 'must have'. The popularity of these shrimps has been based on the erroneous belief of some inexperienced hobbyists that these organisms will keep their reef fishes disease free. If the life support system of the reef display is under-dimensioned, stocked fishes will always be more vulnerable to the deleterious action of pathogens. In these situations, cleaner shrimps will certainly not help to prevent the proliferation of fish diseases. None the less, it is not uncommon to observe fishes seeking the services of cleaner shrimps in reef aquariums. In general, these shrimps set up their 'cleaning stations' near small caves or ledges in the aquascape, vigorously advertising their services through mesmerizing body movements and by the waving of their long (usually white) antennae. The fish 'customer' slowly approaches the cleaner shrimp, allowing it to climb on to its body and inspect the skin, mouth and/or gills using their clawed, multi-articulate, second pair of walking legs. Even voracious fish species, such as groupers and moray eels, are known to seek the services of cleaner shrimps. None the less, cleaner shrimps are not totally safe from fish predation, with certain species of wrasse, and trigger and hawk fishes preying upon them in reef aquariums. This predatory behavior usually seems to be more frequent when adding cleaner shrimps to aquariums that already contain those fish species.

Recently, the new reef aquarium trends promoting 'less technology and more biology' have contributed to the use of certain shrimps to control the growth of nuisance organisms (e.g. glass anemones). The main issue for such 'janitor' species is efficiency. If a shrimp species is able to control pest organisms that may damage other species present in the aquarium (e.g. corals) or that are simply not esthetically attractive, it is not necessary for the shrimp species to be particularly attractive in order for it to successfully enter the aquarium trade. However, if a species controls pest organisms and simultaneously exhibits one of the features mentioned above, particularly an attractive color pattern, it will certainly reach higher market values.

A species is termed 'reef-safe' if it does not damage any other inhabitants commonly present in coral-reef displays. Therefore, even the most colorful shrimp will have a slender chance of becoming a top-selling species if it damages other aquarium organisms, particularly corals and tridacnid clams. Until recently the 'reef-safeness' of an ornamental shrimp was not usually decisive when considering its purchase. Since hobbyists have become increasingly aware, some widely traded ornamental shrimp species have seen their popularity decrease because they are not 'reef-safe'.

1.3 Recruiting new ornamental shrimp species for the marine aquarium trade

The recruitment of new shrimp species for the aquarium industry may be vital in order to minimize current pressure on wild populations of other species already being traded. The more similar the colors and/or behaviors of newly recruited species are to shrimps that are popular to hobbyists, the higher is the possibility of this approach being successful.

Recruiting a new species to the aquarium trade is certainly one of the best ways to create or increase the commercial value of marine organisms. The income generated from 1 kg of organisms from the same species collected for human consumption increases over 100-fold when sold for the aquarium hobby (Wabnitz *et al.*, 2003). Certain shrimp species that are currently discarded as by-catches from food fisheries may also be recruited to the marine aquarium trade and become an added-value item to the fishery. There is an erroneous belief that only marine species from coral reefs are potential targets for the aquarium trade. In fact, several species from estuarine and lagoon areas, as well as those inhabiting subtropical or warm-temperate waters, may display high market potential. One of the main concerns about the collection of ornamental species from habitats other than coral reefs is the lack of suitable legislation regulating the capture of those species. Such situations may endanger wild populations if sustainable collection practices are not enforced.

As in any other hobby, rarities are highly prized by reef aquarium keepers. A species termed as rare in the hobby does not necessarily have to be uncommon in the wild. If a species displays a reduced geographic distribution (e.g. endemic species), or if it occurs in deeper reef areas or in a region in which ornamental species collection is not a commercial activity, it may reach higher market values as a result of its rarity in the trade.

New ornamental shrimp species in the aquarium trade are either enthusiastically accepted by hobbyists or face a sceptical reception and are labelled as 'unsuitable' for reef tanks. As previously noted, the main concern of many hobbyists is whether or not a species is 'reef safe'. It is understandable that certain hobbyists will be reluctant to try an unknown shrimp species in a reef aquarium with highly priced coral pieces. However, if this new ornamental shrimp species is able to control nuisance organisms, hobbyists may be willing to test them in their displays in an attempt to minimize the deleterious effects of pests (e.g. glass anemones, *Aiptasia*). Shrimp species exhibiting robust chelae or associating with corals are less prone to be readily accepted by hobbyists since they are immediately labelled as 'not reef safe'.

Shrimp species displaying unusual feeding habits, and those that will not accept the available commercial diets, may also be less appealing to hobbyists. This unwillingness to accept organisms with uncommon dietary needs is a direct consequence of a growing ethical awareness in the aquarium trade, since most hobbyists now try to avoid ornamental species that they believe will starve in captivity. For this reason, certain obligate symbiotic shrimp species displaying remarkable colors may never be recruited by the industry if their hosts cannot be successfully kept in captivity.

Sometimes, it is only after effective marketing efforts, supported by irrefutable experimental evidence, that a new shrimp species will be promoted to ornamental status and become available commercially.

Ideally, only cultured specimens of new ornamental shrimp species should be available in the market. The main reason to support this approach is that population studies for newly recruited species are usually unavailable. In this way, when a new ornamental species starts to be heavily captured from the wild, it is usually impossible to estimate how harmful that practice may be for the targeted species. If culture protocols are developed before presenting a new ornamental species to the industry, it is possible to supply the market with captive-bred specimens and avoid (or at least minimize) the harvesting pressure on wild populations. Nevertheless, if the demand for specimens of new ornamental species is not entirely fulfilled by cultured organisms, there is always the risk of wild specimens starting to be collected to supply the industry.

1.4 New 'best-selling' marine ornamental shrimps

Recruitment of future shrimp species for the aquarium trade should focus on species that may perform specific 'janitor' tasks in reef aquariums. Hobbyists

have become more sceptical towards the use of chemical products that are claimed to eradicate unwanted algae or nuisance invertebrates without damaging other aquarium organisms. In general, the use of chemical products to control pest organisms is a time-consuming task, is largely inefficient, and too often negatively affects other reef organisms. It is therefore now common in the industry to advocate the use of fish or invertebrate species that may help control or even eradicate reef aquarium pests.

Excessive algae growth is certainly the major problem among inexperienced marine aquarium owners (Sprung, 2002). As a result of either inadequate set-up or the presence of excessive nutrients, it is not uncommon to record reef displays overgrown with unwanted algae (e.g. green hair algae, *Derbesia* spp.). Undesirable algae may cover other organisms in the display, namely highly priced stony corals, damaging them and in extreme cases causing their death. Obviously, using organisms to control this type of situation does not solve the basic problems that lead to the uncontrolled growth of unwanted algae. However, this approach may provide a time window to stop or reduce the propagation of algae, while correcting the initial problems that triggered algal growth. A shrimp species that includes in its feeding regime a large proportion of algae may successfully be recruited for the marine aquarium trade. Although such species may not display beautiful coloration, they will certainly be sold in large numbers as long as they only ingest undesirable algae and do not damage any other organisms in the aquarium.

Other pest organisms that may rapidly proliferate in reef aquariums are the flatworms, *Convolutriloba* spp.. Even though some of these organisms are only unesthetic, some species may be toxic to marine aquarium fishes (Sprung, 2001). A shrimp species that helps to control these flatworms would certainly be in high demand from hobbyists. So far only pricey reef fishes are employed to eradicate these pests, and final results are highly variable.

Recently, some hobbyists have faced serious problems with the involuntary introduction of nudibranch species that feed on certain species of stony corals. The major concerns probably relate to an unidentified species of nudibranch that eats *Montipora* Blainville, 1830. Either wild or cultured colonies of *Montipora* are common vehicles for the introduction of these pests, whose well camouflaged adults or concentric spirals of egg strings are virtually undetectable. Unfortunately for hobbyists, these organisms display direct development (hatching as miniature adults) or fully lecithotrophic swimming larvae (able to use egg yolk reserves to fulfil their energy needs until metamorphosis). A single egg string may give rise to tens, sometimes hundreds, of tiny nudibranchs, which readily devour large colonies of *Montipora*. Although some reef fishes seem to be able to eat these nudibranchs, hobbyists would gladly test ornamental shrimp species that could control these organisms, since shrimps could easily reach the crevices in which nudibranchs commonly hide so as to avoid fish predation.

A remarkable species that would require a special display tank is the semi-terrestrial shrimp of the genus *Merguia* Kemp, 1914. These shrimps are known

to commonly climb mangrove roots, remaining outside the water for long periods of time (Abele, 1970; Gillikin *et al*., 2001).

In conclusion, the permanent need for novelty in the aquarium hobby will certainly allow new shrimp species to be recruited for the industry. Poorly studied and sometimes even unknown shrimp species will quickly gain popularity and become highly priced in the aquarium trade. All those involved in the industry must ensure that sustainability remains a top priority and that the marine aquarium hobby actively contributes to the promotion and preservation of coral reefs.

Chapter 2

Collected and Traded Species

2.1 Collecting sites and techniques

Marine ornamental shrimps available in the aquarium trade are mainly collected in the Indo-Pacific region, as well as in the Caribbean. *Lysmata* spp. and *Stenopus* spp. are among the top 10 of the most heavily collected marine ornamental invertebrates (excluding corals), with Indonesia, the Philippines and Sri Lanka being the main exporting countries (Wabnitz *et al.*, 2003). However, the limited amount of reliable data on imports and exports of marine ornamental shrimps means that the numbers commonly presented in official reports addressing the trade of these highly priced organisms are certainly underestimated. As an example, the data presented by Wabnitz *et al.* (2003) (according to the Global Marine Aquarium Database, GMAD), state that a total of about 107 000 shrimps from several *Lysmata* species were exported worldwide during 1998 to 2003. In a single year, the number of peppermint shrimps *Lysmata* spp. being collected and traded solely in the USA is probably higher than this value (A.L. Rhyne, pers. comm.). In this way, the real number of marine ornamental shrimps being collected from coral reefs each year worldwide may be up to 10 times higher than is currently reported. Because of the increasing global popularity of marine aquarium keeping, it is expected that an increase in the collection of marine ornamental shrimps, as well as a diversification of commonly exported species, will occur in the next few years.

Destructive fishing techniques commonly employed to capture marine ornamental fishes in Southeast Asia, such as the use of cyanide (Jones *et al.*, 1999; Halim, 2002), are not suitable for the collection of marine ornamental shrimps. Obviously, collectors targeting marine ornamental shrimps do not employ iron crowbars, hammers or chisels, although these are commonly used to collect corals or other sessile invertebrates (Lovell, 2001). Most marine ornamental shrimps are collected by divers (using more or less advanced diving gear) equipped with small tubular hand nets and a slim rod. This rod is sometimes

referred to as a 'tickling stick' and is used to 'tickle' shrimps out of their holes or crevices and into the nets, which are strategically placed at the entrance of the shrimp's shelter. Captured shrimps are often placed in small, perforated, transparent, plastic containers and stocked in inland facilities prior to shipping. However, when ornamental species are harvested in isolated collection sites, they may remain on the fishermen's boats for several days before being landed, which may eventually promote higher mortality rates as a result of unsuitable handling practices (Baquero, 1995).

Occasionally, marine ornamental shrimps may become a valuable by-catch for some food fisheries. In Florida, peppermint shrimps *Lysmata* spp. have changed from a wasted by-catch to a valuable income source for fishermen using traps to collect stone crabs or spiny lobsters, as well as for trawlers fishing for bait shrimp.

2.2 Marine ornamental shrimp families

In the present work, the distribution areas and maximum body sizes given for each ornamental shrimp species are after Debelius (2001). The taxonomic classification followed is that proposed by Martin & Davis (2001) (Table 2.1).

Marine ornamental shrimps currently traded in the aquarium hobby mainly belong to the following families: Stenopodidae Claus, 1872; Alpheidae Rafinesque, 1815; Gnathophyllidae Dana, 1852; Hippolytidae Dana, 1852; Hymenoceridae Ortmann, 1890; Palaemonidae Rafinesque, 1815; and Rhynchocinetidae Ortmann, 1890 (Figure 2.1). Although highly important in food fisheries and aquaculture, penaeid shrimps (Dendrobranchiata Bate, 1888) have never experienced much popularity in the hobby. All commonly traded ornamental shrimps belong to the group of decapod crustaceans that incubate their embryos under the abdomen: the Pleocyemata Burkenroad, 1963.

2.2.1 Family Stenopodidae

Curiously, some of the most heavily collected and traded shrimps are not actually true shrimps. Shrimps of the genus *Stenopus* Latreille, 1819, commonly referred to as boxer shrimps, are some of the most emblematic species available in the hobby and differ from true shrimps in several morphological aspects. These organisms belong to the infraorder Stenopodidea Claus, 1872, and commonly occur as mated pairs and can be easily distinguished from 'true shrimps' by the presence of chelae (claws) in the third pair of pereiopods (walking legs). Although their taxonomic position among decapod crustaceans is still being debated, there is strong evidence that stenopodidean shrimps may be the closest ancestors of true shrimps. Stenopodidean species present in the aquarium trade are all members of the family Stenopodidae, being free living

Table 2.1 Families, scientific and common names, world distribution, availability in the trade and retail value of marine ornamental shrimps

Family	Species	Common name	Distribution	Availability	Retail value
Stenopodidae	*Stenopus cyanoscelis*	Blue-legged boxing shrimp	Tropical West Pacific	Average	High[3]
	Stenopus hispidus[1]	Banded boxing shrimp	Circumtropical	High	Average[3]
	Stenopus pyrsonotus	Ghost boxing shrimp	Indo-Pacific	Low	High[3]
	Stenopus scutellatus	Golden boxing shrimp	Western Atlantic, common in the Caribbean	High (USA) Low (EU)	Average[3] (USA) High[3] (EU)
	Stenopus spinosus	Mediterranean boxing shrimp	Eastern Atlantic; common in the Mediterranean[2]	Low	High[3]
	Stenopus tenuirostris	Blue boxing shrimp	Indo-Pacific	Average	Average[3]
	Stenopus zanzibaricus	Zanzibar boxing shrimp	Indo-Pacific	Average	Average[3]
Alpheidae	*Alpheus ochrostriatus*	Fine-striped pistol shrimp	Indo-Pacific	Average	High[4]
	Alpheus randalli	Randall's pistol shrimp	Western Pacific	Low	High[4]
Gnathophyllidae	*Gnathophyllum americanum*	Bumblebee shrimp	Circumtropical	Low	Low[5]
Hippolytidae	*Lysmata amboinensis*[1]	Skunk cleaner shrimp	Indo-Pacific	High	Average
	Lysmata ankeri	Peppermint shrimp	Western Atlantic	High	Low (USA)[6] Average (EU)
	Lysmata boggessi	Peppermint shrimp	Western Atlantic	High	Low (USA)[6] Average (EU)
	Lysmata californica	Peppermint shrimp	Eastern Pacific	High	Low (USA)[6] Average (EU)
	Lysmata debelius[1]	Fire shrimp	Indo-Pacific	High	High
	Lysmata grabhami	Lady cleaner shrimp	Atlantic	Low	High
	Lysmata kuekenthali	Peppermint shrimp	Indo West Pacific	Average	Average
	Lysmata rathbunae	Peppermint shrimp	Western Atlantic	High	Low (USA)[6] Average (EU)
	Lysmata seticaudata	Monaco shrimp	Eastern Atlantic	Average	Average (EU only)[7]
	Lysmata vittata	Peppermint shrimp	Indo West Pacific	Average	Low (USA)[6] Average (EU)

Continued

Table 2.1 (Continued)

Family	Species	Common name	Distribution	Availability	Retail value
Hippolytidae (Continued)					
	Lysmata wurdemanni[1]	Peppermint shrimp	Western Atlantic	High	Low (USA)[6] Average (EU)
	Lysmatella prima	—	Indo-Pacific	Low	High
	Parhippolyte mistica	Candy cane shrimp	Indo-Pacific	Low	High
	Saron marmoratus	Marbled shrimp	Indo-Pacific	Moderate	Low[8]
	Saron neglectus	Green marbled shrimp	Western and Central Pacific	Moderate	Low[8]
	Saron rectirostris	Purple-legged marble shrimp	Western Pacific	Moderate	Low[8]
	Thor amboinensis	Sexy shrimp	Circumtropical	High	Moderate
Hymenoceridae	*Hymenocera elegans*	Harlequin shrimp	Indo West Pacific	Moderate	High[3]
	Hymenocera picta	Harlequin shrimp	Central and Eastern Pacific	Moderate	High[3]
Palaemonidae	*Periclimenes brevicarpalis*	White-patched partner shrimp	Indo-Pacific	Moderate	Moderate
	Periclimenes pedersoni	Pederson's partner shrimp	Western Atlantic, common in the Caribbean	Moderate	Moderate
	Periclimenes yucatanicus	Yucatan partner shrimp	Western Atlantic, common in the Caribbean	Moderate	Moderate
	Periclimenes holthuisi	Holthuis's partner shrimp	Indo West Pacific	Moderate	Moderate
	Urocaridella antonbruunii	Bruun's cleaner shrimp	Indo West Pacific	Moderate	Moderate[9]
Rhynchocinetidae	*Rhynchocinetes durbanensis*	Durban dancing shrimp	Indo-Pacific	High	Low[10]

[1] The most popular ornamental shrimp species in the marine aquarium hobby.
[2] Boxing shrimps morphologically similar to Eastern Atlantic specimens have also been collected in the Gulf of Mexico and Brazil.
[3] Reach higher retail prices when sold as mated pairs.
[4] Only reach high retail prices when sold with their symbiotic partner gobies.
[5] Its low retail value is a consequence of its reduced body size.
[6] The low retail value of these shrimps in the USA is a result of their being a commonly sold by-catch of lobster and crab fisheries.
[7] Only traded in Europe as an alternative to peppermint shrimps. Cultured specimens are commonly available.
[8] Despite their attractive appearance these shrimps reach low retail values because they are not reef safe.
[9] After being scientifically documented as a fish cleaner its retail price has been rapidly increasing.
[10] The low retail value is probably a result of the fact that these organisms are highly available in the trade (they occur in the wild in large aggregations, which simplifies their collection) and are not considered as reef-safe by most hobbyists.

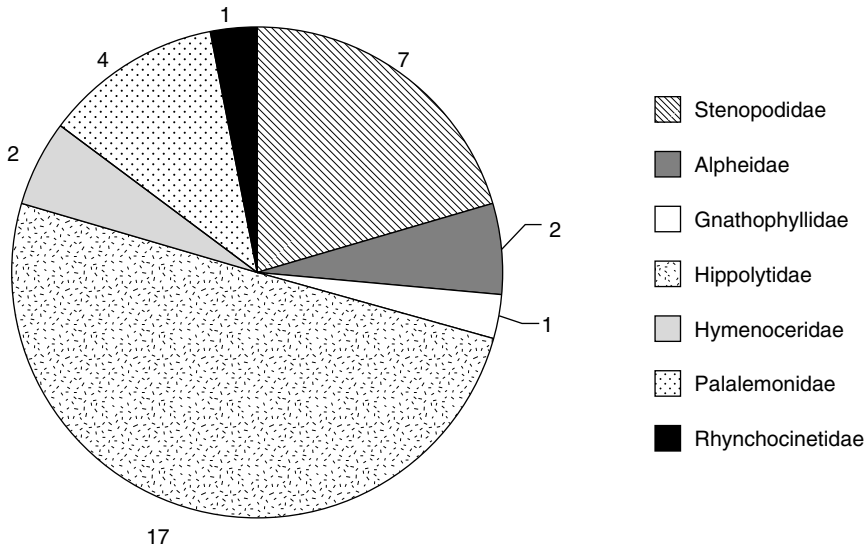


Figure 2.1 Ranking of most traded marine ornamental shrimp species distributed by their families.

and inhabiting relatively shallow water. The deeper water and sponge-associated species of the family Spongicolidae Schram, 1986 (see Saito & Takeda, 2003 for details) are rarely seen in the hobby. The most popular stenopodidean species in reef aquariums is certainly *Stenopus hispidus* (Oliver, 1811), also known as the barber pole or coral banded boxing shrimp. This circumtropical species is present in all tropical seas of the world, being mainly collected for the aquarium trade in the Caribbean, Eastern Africa, Southeast Asia and Hawaii. It may reach up to 8 cm and displays a distinctive body coloration: a white body with conspicuous orange transverse bands in the abdomen and larger chelipeds (pincers), while the basal area of the pereiopods exhibits a bluish hue (see color plate 1). It has regularly been recorded as a 'fish cleaner', and is commonly observed 'cleaning' moray eels. Another large sized stenopodidean (up to 10 cm) regularly available in the aquarium trade is the ghost boxer shrimp *Stenopus pyrsonotus* Goy and Devaney, 1980. This species inhabits the tropical waters of the Indo-Pacific, being fairly well known from Hawaii. Its bright red longitudinal stripe on the dorsal region and thin elongated chelipeds make it unique among *Stenopus* species. Its white antennae may reach a length of about 20 cm. *Stenopus cyanoscelis* Goy, 1984 occurs in the tropical west Pacific and reaches up to 4 cm. It has a yellowish body and bluish legs and exhibits red and white bands in the abdomen and chelipeds. Like many other *Stenopus*, it has long white antennae. The small sized *Stenopus tenuirostris* De Man, 1888 reaches only 2 to 3 cm in body length and is one of the smallest species in the genus. It is distributed throughout the Indo-Pacific, and is easily recognized by the orange bands on its abdomen and chelipeds

and by the bluish tone of its cephalothorax (carapace). Females of this species also display bright blue ovaries, which may eventually be observed developing in the dorsal region of the cephalothorax under the carapace. Newly extruded embryos of *S. tenuirostris* are also bright blue, and are present in the ventral portion of the female's abdomen during the incubation period. *Stenopus zanzibaricus* Bruce, 1976 inhabits the Indo-Pacific, and is well known from Kenya to Micronesia. It can reach up to 3 cm and its bright yellow body and first leg segments, along with its red antennae, make it distinct from other *Stenopus* species also displaying orange/red and white transversal bands. The golden boxer shrimp *Stenopus scutellatus* Rankin, 1898 is widely collected throughout the Caribbean and may reach 4 cm in body length. It can be identified by its golden yellow body. Additionally, it displays orange and white bands on the chelipeds and on the posterior end of the abdomen. Recently, several specimens morphologically similar to the Eastern Atlantic and Mediterranean *Stenopus spinosus* (Risso, 1827) have been collected in the Gulf of Mexico and Brazil. This unexpected occurrence in the Western Atlantic is still being investigated by researchers, but collected specimens have already entered the aquarium trade. Their novelty in the American market means that these specimens are commanding higher market values among hobbyists, although they have been commonly used by European aquarium keepers recreating Mediterranean biotopes. *Stenopus spinosus* is commonly recorded in shallow areas in the Atlantic Islands and the Mediterranean, occupying deeper waters in the coastal waters of the Eastern Atlantic. It may reach up to 8 cm in body length, presents a yellow–orange body, white-tipped chelipeds, and a red-tipped telson and uropods (central and lateral appendages at the posterior end of the abdomen, respectively) (see color plate 2).

2.2.2 Family Alpheidae

The majority of marine ornamental shrimp species regularly available in the marine aquarium trade belong to the infraorder Caridea Dana, 1852, being easily distinguishable from stenopodideans by the absence of chelae in the third pair of pereiopods (Figure 2.2).

Shrimps belonging to the family Alpheidae are commonly designated as pistol or snapping shrimps. This family includes more than 600 species, distributed among at least 36 genera. However, only members of the genera *Alpheus* and *Synalpheus* are commonly available in the aquarium industry. The alpheid frontal region of the carapace is unique among decapod crustaceans, with most species displaying their eyes dorsally covered by anterior projections of the carapace that are termed orbital hoods. Orbital hoods vary among groups of alpheid shrimps and are of great taxonomic importance for researchers (Banner & Banner, 1983). A remarkable feature displayed by some alpheid shrimps is the existence of a modified claw in their first pair of pereiopods, which exhibits a complex snapping mechanism (Anker

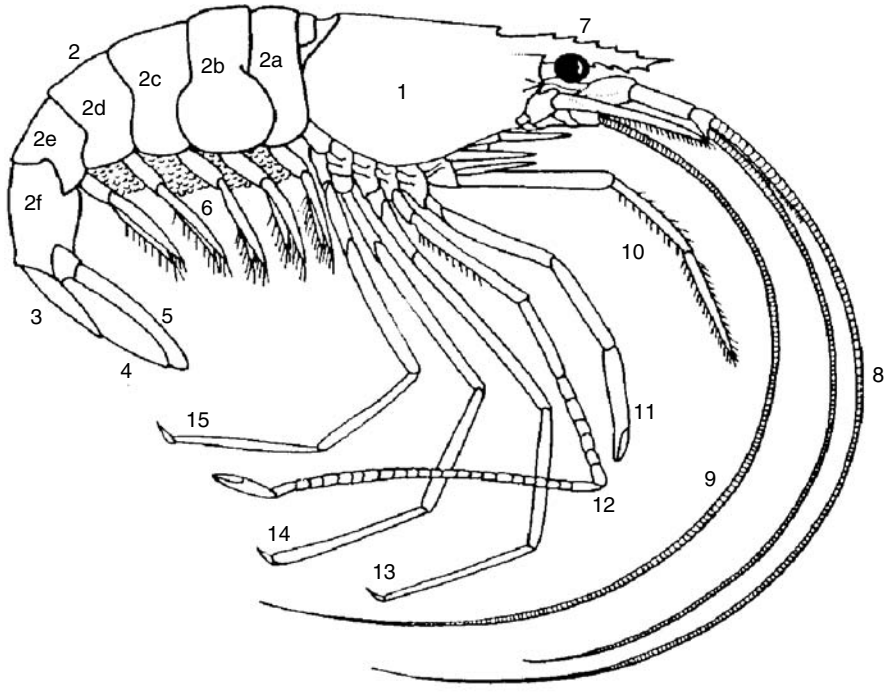


Figure 2.2 Morphological details of a 'typical' adult caridean shrimp: 1, Carapace or cephalothorax; 2, abdomen; 2a, first abdominal segment; 2b, second abdominal segment (note how the round pleura overlap the ones from the first and third abdominal segments); 2c, third abdominal segment; 2d, fourth abdominal segment; 2e, fifth abdominal segment; 2f, sixth abdominal segment; 3, telson; 4, inner branch of the uropods; 5, outer branch of the uropods; 6, pleopods (note how developing embryos are carried under the abdomen and attached to the pleopods); 7, rostrum; 8, antennules; 9, antenna; 10, third maxilliped; 11, first pereiopod (due to the presence of a chela it is also termed a cheliped); 12, second pereiopod (also displaying a chela); 13, third pereiopod (unlike stenopodideans, caridean shrimps do not exhibit chelae on these appendages); 14, fourth pereiopod; 15, fifth pereiopod.

et al., 2006). This snapping claw is a key innovation (*sensu* Mayr, 1960) among decapod crustaceans and a multifunctional appendage, being used for either defense or aggression in interspecific interactions. The loud sound produced by the modified claw of alpheid shrimps can be easily heard in marine aquariums and some inexperienced hobbyists are sometimes reluctant to keep these organisms in their displays. The main reason for this pistol shrimp avoidance is that the snapping sound produced greatly resembles the worst possible sound for an aquarium keeper — the sound of cracking glass! Nevertheless, several alpheid species are still very popular in the hobby, namely the ones displaying symbiotic behavior. Pistol shrimps are known to associate with different types of invertebrates, such as sponges, cnidarians, echiurans, molluscs, other crustaceans and echinoderms (Anker *et al.*, 2001, 2005; Marin *et al.*, 2005). Unfortunately, the fascinating

behavior of many of these species, namely the sponge-dwelling eusocial shrimps of genus *Synalpheus* (Duffy, 1996; Tóth & Duffy, 2005), cannot yet be properly admired in captivity. The main obstacles to the display of these remarkable species are the technical constraints related to the life support systems required to house the shrimps' host species. In addition to invertebrate organisms, alpheid shrimps are also known to associate with gobiid fishes (Karplus, 1987; Thompson *et al.*, 2005). These associations are common in Indo-Pacific coral reefs, with approximately 120 described species of gobies and 30 species of shrimp being recorded as somehow interacting mutualistically (Karplus, 1987). Alpheid species which associate with gobiid fishes are regularly imported and available in the aquarium trade. Shrimps sold paired with their partner gobiid achieve higher market values than if sold alone. Additionally, mated pairs of these alpheid shrimps also reach higher prices. *Alpheus randalli* Banner, 1981 is a highly priced and distinctive species because of its yellow walking legs, the red and white bands covering its body and its enlarged chelipeds. It has been recorded in association with gobies from four different genera: *Amblyeleotris* Bleeker, 1874; *Stonogobiops* Polunin and Lubbock, 1977; *Tomiyamichthys* Smith, 1956; and *Flabelligobius* Smith, 1956 (Debelius, 2001). It occurs in the Western Pacific and may reach up to 3 cm. *Alpheus ochrostriatus* Miya is also known as the fine striped snapping shrimp, displaying fine pinkish longitudinal stripes over its body and a white blotch in the first and fourth abdominal segments. This species lives in association with the beautiful goby *Cryptocentrus cinctus* (Herre, 1936) and commands high market values. It is present in the Indo-Pacific and reaches a total length of 5 cm.

2.2.3 Family Gnathophyllidae

Shrimps of the family Gnathophyllidae commonly display small, stout bodies. Owing to their reduced size and/or associative behavior (mainly with echinoderms), hobbyists tend to avoid most members of this family, since they can only be properly displayed in specially designed aquariums. In this way, only *Gnathophyllum americanum* Guérin-Méneville, 1856 is usually available in the aquarium trade. This gnathophyllid shrimp is also popularly known as the bumblebee shrimp. It reaches up to 3 cm in body length and displays a unique pattern of white, yellow and black thin transverse lines over its entire body. It has already been recorded in all tropical seas, and the pattern displayed by its transverse lines appears to be unique for each specimen. Members of the genus *Gnathophyllum* Latreille, 1819 have an unmistakable appearance, since their head appears to have been cut off!

2.2.4 Family Hippolytidae

Shrimps belonging to the family Hippolytidae are probably the most popular ornamental shrimps among aquarium hobbyists (Figure 2.3). This

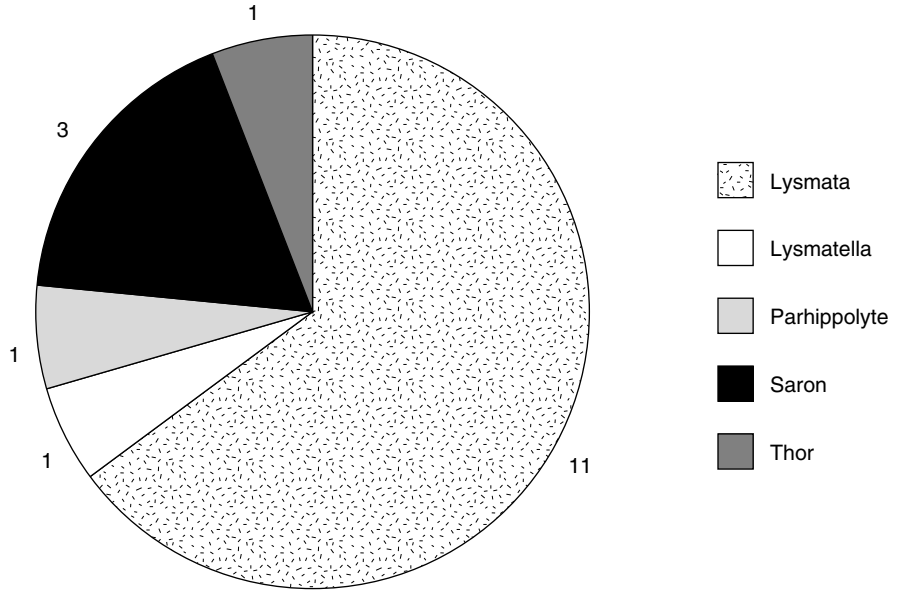


Figure 2.3 Number of most traded marine ornamental shrimp species in the family Hippolytidae distributed by their genera.

popularity is largely due to the shrimps in the genus *Lysmata* Risso, 1816, commonly known as peppermint and cleaner shrimps. All peppermint shrimps display average body sizes ranging between 3.5 and 6 cm. The so-called peppermint shrimps belong to a large number of distinct *Lysmata* species, although several of them may be closely related. Recently, Rhyne & Lin (2006) have identified a Western Atlantic *wurdemanni* species complex, reporting the existence of at least five different species: *Lysmata ankeri* Rhyne and Lin, 2006, *Lysmata bahia* Rhyne and Lin, 2006, *Lysmata boggessi* Rhyne and Lin, 2006, *Lysmata rathbunae* Chace, 1970, and *Lysmata wurdemanni* (Gibbes, 1850). Despite species-specific variations in their coloration patterns, all peppermint shrimp species generally exhibit semi-translucent bodies with longitudinal, transverse, and/or oblique red bands. Certain species display a more or less intense blue tinge on the posterior ends of their telson and uropods. All these species are generally labelled as '*wurdemanni*' in the trade and are commonly used in marine aquariums to control the outbursts of glass anemones *Aiptasia*. Indo-West Pacific species of peppermint shrimps, *Lysmata kuekenthali* (De Man, 1902) and *Lysmata vittata* (Stimpson, 1860), as well as the cooler water species from the Eastern Pacific, *Lysmata californica* (Stimpson, 1866), also display more or less similar body colorations to those recorded in the Caribbean and are also available in the aquarium trade as *Aiptasia* eaters. The Monaco shrimp *Lysmata seticaudata* (Risso, 1816) inhabits the warm temperate waters of the Mediterranean and the North-eastern Atlantic. It presents similar color patterns to other peppermint shrimps and only recently has it become available in the trade as a suitable

species for reef aquariums and the control of glass anemones. The recruitment of this ornamental species to the marine aquarium industry was in a sense unique since it was the first time that a large number of cultured specimens from a new species entered the trade before wild ones.

Despite the popularity that peppermint shrimps may have among hobbyists, cleaner shrimps of the genus *Lysmata* are certainly the trademark of ornamental shrimps in the aquarium industry. *Lysmata amboinensis* De Man, 1888 and *L. grabhami* (Gordon, 1935) are also known respectively as the skunk cleaner shrimp and the lady cleaner shrimp. While *L. amboinensis* is present in the Indo-Pacific, the Red Sea and Hawaii, *L. grabhami* occurs on both sides of the tropical and subtropical Atlantic Ocean. Both species display large and conspicuous white antennae, as well as a third, white pair of maxillipeds. Their legs and bodies are bright yellow, with a longitudinal white stripe running from head to tail on the dorsal surface, bordered on each side by a blood red longitudinal stripe. Despite presenting remarkably similar morphology, both species can be distinguished *in vivo* by the coloration pattern of their telson and the external branch of the uropods: *L. amboinensis*'s longitudinal white stripe is discontinued in the telson, forming an inverted white triangle in the posterior portion of this body segment, and each external branch of the uropods exhibits two distinctive white patches on their external margins (see color plate 3); *L. grabhami*'s longitudinal white stripe is continuous, running from the anterior tip of the rostrum to the posterior end of the telson, and each external branch of the uropods displays a continuous white line on the outer margins. Both species may display body sizes ranging up to 6 cm, although specimens available to hobbyists are commonly smaller. Although *L. amboinensis* is highly traded in the aquarium industry, *L. grabhami* is rarely available, thus commanding higher market values. Both species have been commonly observed 'cleaning' numerous fish species, from groupers to moray eels, both in the wild and in reef aquariums.

Another hobbyist 'must have' is the fire shrimp *Lysmata debelius* Bruce, 1983, a species occurring from the Indian Ocean to the Central Pacific. It displays brilliant red body coloration and conspicuously white antennae and terminal segments of the third to fifth walking legs (see color plate 4). Additionally, *L. debelius* also displays several large white dots on their cephalothorax and pleopods. Specimens displaying white dots on the dorsal surface of their abdomen have been recognized as belonging to a different species named *Lysmata splendida* Burukovski, 2000. Both these species are closely related, although *L. splendida* is rarely available in the aquarium trade and is only known from its type locality, the Ary Atoll in the Maldives (Burukovski, 2000). Both species may reach up to 5 cm in total length.

Although rarely available in the aquarium trade, *Lysmatella prima* Borradaile, 1915 has started to be highly appreciated by reef aquarium keepers. This species is closely related to the members of the genus *Lysmata*, even though both genera can be readily separated by the absence of epipods on the

pereiopods of *Lysmatella* (Chace, 1997) [epipods are specialized structures commonly involved in gill cleaning behavior inside the branchial chamber (Bauer, 1979, 1981a)]. It is a small species (2–3 cm in body size) and displays white to yellowish legs and long antennae, with longitudinal chocolate brown stripes along its body. *Parhippolyte mistica* (J. Clark, 1989), also known as candy cane shrimp, is occasionally available for trade. It is a cryptic species commonly present in Indo-Pacific marine caves. Its third to fifth pereiopods are white and remarkably long and its abdomen and tail fan show diagnostic intense vertical red bands. The antennae are also remarkably long, being red in their anterior tip and becoming progressively brilliant white towards their posterior end. Due to their long pereiopods, *P. mistica* appears to gently hover over the bottom. It can reach up to 4 cm in body length, although it appears somewhat larger owing to its long pereiopods and antennae.

Marbled shrimps belong to the genus *Saron* Thallwitz, 1891, a group of nocturnal organisms that commonly live among coral rubble and display mimetic coloration and bush-like setae covering their bodies. *Saron* shrimps display strong sexual dimorphism, with males exhibiting not only an enlarged first pair of chelipeds but also an enlarged third pair of maxillipeds (Tirmizi & Kazmi, 1971). Because of their problematic systematics, marbled shrimps are commonly separated in two informal divisions: the *marmoratus* and the *neglectus* groups (Debelius, 2001). Despite having earned a reputation of not being reef-safe (they may prey upon other aquarium organisms, namely corals and tridacnid clams (Sprung, 2001; Shimek, 2004)), their amazing coloration still gains them some popularity among hobbyists.

The most commonly available member of the genus *Saron* in the aquarium trade is the marbled shrimp *Saron marmoratus* (Olivier, 1811), a species inhabiting the Indo-Pacific from East Africa to Hawaii. It displays patches of various brownish hues over the entire body, giving a marbled look; there are also numerous bush-like setae on the body. It may reach a total length up to 5 cm and is the nominal species of the *marmoratus* group.

The green marbled shrimp *Saron neglectus* De Man, 1902 is characterized by its greenish coloration and by the presence of large ocelli on the abdomen and the telson. The species is present in the West and Central Pacific, may reach up to 4 cm and is the nominal species of the *neglectus* group.

Saron rectirostris Hayashi, 1984 is commonly known in the trade as purple-legged marble shrimp and occurs in the Western Pacific. It resembles a piece of dead coral, exhibiting a cream-like coloration dotted with brown pore-like spots. This species is easily recognized by its massive rostrum, which displays numerous rostral teeth in its dorsal and ventral margin. The pereiopods and tail fan show an intense purple coloration and it reaches a body size of approximately 3 cm.

The sexy shrimp *Thor amboinensis* (De Man, 1888) is one of the few truly circumtropical shrimp species, being present in all tropical seas of the world. Despite its small size (adult specimens only reach 2 cm), *T. amboinensis* is

very popular in the aquarium trade because of its associative behavior with numerous species of sea anemones and its habit of stretching and waving the abdomen upwards. The reason for this unusual behavior is still unknown. Sexy shrimps commonly display transparent brownish bodies dotted with large iridescent white dots bordered by a thin blue line. Females are commonly twice the size of males.

2.2.5 Family Hymenoceridae

Ornamental shrimps commonly designated as harlequins are currently placed in the family Hymenoceridae, though older literature refers to them as members of the Gnathophyllidae. Arguably, these shrimps are some of the most striking decapods in the world, showing unique morphological features and coloration patterns. They are readily recognized by divers and aquarium keepers, and have been compared to orchids and mounted medieval knights in ornate armour (Debelius, 2001). Members of the genus *Hymenocera* Latreille, 1819 display greatly expanded and leaf-like appendages, namely their third maxillipeds, the outer flagellum of the antennules and the fixed fingers of the second pair of chelipeds. Currently, two closely related species with remarkably similar morphology are recognized in the genus: *Hymenocera elegans* Heller, 1861, occurring in the Red Sea from East Africa to Indonesia and northern Australia; and *H. picta* Dana, 1852, a species inhabiting the Central and Eastern Pacific. The main feature separating these two species is their body coloration: *H. elegans* shows a cream or white body with large brownish-purple blotches surrounded by bright blue margins (see color plate 5); *H. picta* also exhibits a cream or white body but is covered with wine-red and pinkish blotches with yellowish margins. Although several authors question the validity of coloration patterns to discriminate these two different species, it must be noted that they do occur in different geographic areas and that specimens displaying intermediate coloration patterns have never so far been recorded. Female harlequin shrimps are commonly larger than males and exhibit large colored blotches in the ventral region of their abdominal segments. Both *Hymenocera* species may reach up to 5 cm and feed exclusively on starfishes. Because of their monogamic behavior and agonistic behavior towards conspecifics of the same sex, a mated pair of harlequin shrimps attains higher market values than individuals being sold individually.

2.2.6 Family Palaemonidae (subfamily Pontoniinae)

The most highly demanded shrimps for marine aquariums in the family Palaemonidae are the partner shrimps of genus *Periclimenes* Costa, 1844. This shrimp genus belongs to the subfamily Pontoniinae Kingsley, 1878 and, like

most other genera in this subfamily, displays associative behavior with a large number of invertebrate groups (cnidarians, molluscs and echinoderms), as well as fishes. In fact, because of the obligate nature of some of these associations and the technical obstacles to keeping certain invertebrate hosts in captivity, many *Periclimenes* species cannot be kept in marine aquariums (e.g. *P. imperator* A. J. Bruce, 1967 living in association with the Spanish dancer nudibranch *Hexabranchus sanguineus* (Ruppell and Leuckart, 1828)).

Two of the most popular partner shrimps occur in the Caribbean: *P. pedersoni* Chace, 1958, and *P. yucatanicus* (Ives, 1891). The first is considered the most well known cleaner species in the Caribbean, and is commonly seen 'cleaning' inside the mouth and gills of large groupers. It is a small species, reaching only 2.5 cm, and lives in association with a variety of sea anemones. It displays conspicuous white antennae and a transparent body with a long and thin V-shaped purple line on the dorsal surface of the abdomen. Its chelipeds show a diagnostic pattern of purple and white transverse bands. *Periclimenes yucatanicus* displays a transparent body with large white blotches on its dorsal surface and conspicuous large blue dots on the tail fan, as well as transverse blue bands on its walking legs. It has already been recorded associating with several sea anemones, jelly fish and corallimorphs. It may reach a total length of 3 cm, but is usually smaller. Despite their popularity, these two *Periclimenes* species are not particularly resilient in captivity, commonly enduring only a few months in marine aquariums (Spotte & Bubucis, 1997).

Periclimenes brevicarpalis (Schenkel, 1902) is also a heavily traded partner shrimp, mainly due to the larger body size displayed by adult specimens (it can reach up to 4 cm). It occurs from the Red Sea to Indonesia and Australia. It displays a transparent body with large white dots, the largest one being located in the dorso-posterior region of the carapace. It also displays five bright orange spots bordered by a thick black line on the tail fan forming conspicuous ocelli. Although in nature it associates with several sea anemones, namely *Cryptodendrum adhaesivum* Klunzinger, 1877, it can be easily kept in a marine aquarium, where it will readily associate with other hosts, or simply seeks shelter among small rocks.

Several species of *Periclimenes* display similar colorations and are usually mislabelled in the aquarium industry as *Periclimenes holthuisi* Bruce, 1969. The true members of this species reach up to 2.5 cm, occur from East Africa to Australia and display a transparent body with white and brownish marks in the chelipeds, the mid-dorsal region of the abdomen and the tail fan. Another species commonly mistaken for *P. holthuisi* is *Periclimenes tosaensis* Kubo, 1951, differing from it mainly in the coloration of its abdominal hump (being white and violet). It is much more common than the former species, occurs from the Maldives to the South China Sea and Japan and also reaches 2.5 cm in body length. It is usually recorded in groups of several individuals and associates with several hosts, namely bubble corals *Plerogyra* Milne-Edwards and Haime, 1848, and mushroom corals *Heliofungia* Wells, 1966. These two *Periclimenes*

species, as well as several others which are morphologically similar, are probably part of a large species complex in the Indo-West Pacific that remains to be properly investigated (Debelius, 2001).

In general, male partner shrimps are usually smaller than females and do not exhibit colored blotches in the lateral-inferior area of their abdominal segments.

2.2.7 Family Palaemonidae (subfamily Palaemoninae)

In recent years another group of remarkable shrimps has started to be traded in the aquarium industry: the members of the genus *Urocaridella* Borradaile, 1915. These shrimps belong to the subfamily Palaemoninae Rafinesque, 1815. Unlike partner shrimps, *Urocaridella* species display a very long rostrum and their transparent bodies are covered by numerous, small, red, white, yellow or brown blotches. Although most specimens traded are commonly sold as *Urocaridella antonbruunii* (A. J. Bruce, 1967), the taxonomy of *Urocaridella* species is still unclear. The only well described species, *U. antonbruunii*, which occurs in the Indo-West Pacific, is often incorrectly referred to as *Leandrites cyrtorhynchus* Fujino and Miyake, 1969. Despite being one of the few shrimp species scientifically recognized as a fish cleaner (Becker & Grutter, 2004, 2005), *U. antonbruunii* does not display the conspicuous long white antennae possessed by other shrimps also labelled fish cleaners (Côté, 2000). They commonly swim by vigorously beating their pleopods when moving towards their fish clients. This behavior gives them the strange appearance of hovering in mid-water.

2.2.8 Family Rhynchocinetidae

Members of the family Rhynchocinetidae are popularly known as hinge beak shrimps. However, their unusual way of moving, resembling tango dancers, has also granted them the popular name of dancing shrimps. Unlike other shrimps, they are able to move the rostrum up and down because of the presence of a flexible joint with the carapace. There are numerous species in the family, which belong to two closely related genera: *Rhynchocinetes* H. Milne Edwards, 1837, and *Cinetorhynchus* Holthuis, 1995. All members of both genera display large black eyes. Although other dancing shrimps may occasionally enter the aquarium trade, the most commonly available species is *Rhynchocinetes durbanensis* Gordon, 1936. This species was erroneously traded for many years as *R. uritai* Kubo, 1942, and can be easily diagnosed by its coloration: red and white lines on a translucent body, also displaying numerous white ocelli and a Y-shaped white mark on the dorso-anterior portion of the carapace. The species displays strong sexual dimorphism, with dominant males exhibiting the first pair of chelipeds greatly enlarged. It is present in the Indo-Pacific region and may reach up to 4 cm in body length.

Chapter 3

Ecological Aspects

3.1 Ornamental shrimp coloration

The majority of traded marine ornamental shrimps are collected from coral reefs. Like reef fishes, caridean and stenopodidean shrimps also display dazzling colorations. However, for many years, reef shrimps were only known from museum collections, which held preserved specimens that had lost their colors. Only after the advent of scuba diving and underwater photography was the true beauty of these organisms truly revealed. The dazzling colorations of most shrimps contrast with the near transparency of others which appear to be able to 'hide in plain sight' (Johnsen, 2001). Color patterns displayed by ornamental shrimps are quite stable over vast geographical areas (Bruce, 1975), although more or less significant changes may occur according to age and sex (Noël, 1983). These chromatic changes are termed morphological and result from the appearance of chromatophores in areas where they are not normally present. In contrast to the long period of time required for morphological chromatic changes to take place, physiological chromatic changes are very fast events, taking from a few seconds to several hours (Noël, 1983). During the diurnal and nocturnal periods, pronounced differences in a shrimp's color intensity and pattern result from the action of morphologically and physiologically different chromatophores (Chassard-Bouchaud & Couturier, 1968, 1969). The role of eyestalks, and consequently the glands present in these structures, is of major importance in the shrimp's capacity to regulate chromatic changes, since the ablation of these structures induces the appearance of abnormal coloration (Chassard-Bouchaud, 1965). It is also clear that the central nervous system plays a major role in the control of chromatic changes, namely through the action of a dispersive and retractile hormone on chromatophores (Noël, 1981).

Marine decapods are predominantly vulnerable to visual predation, usually exerted by fishes, during the settlement period and early juvenile stages (Wahle & Steneck, 1992). These predation-mediated mechanisms play

an important role in the demographic balance of decapod populations. An effective antipredatory strategy employed by these organisms is visual crypsis (Palma & Steneck, 2001). Small, newly settled specimens exhibit colorations that mimic the surrounding environment, minimizing visual detection by potential predators (Endler, 1978; Hacker & Madin 1991; Palma *et al*., 2003). Another common strategy of juvenile shrimps to avoid visual hunting predators is to remain transparent (Bauer, 1981b), a feature that can be well observed during the ontogeny of *Periclimenes* (Noël, 1983).

The highly conspicuous colorations displayed by harlequin shrimps *Hymenocera* may act as a warning to potential predators (Bruce, 1975). Although, in their natural environment, as well as in aquarium displays, fishes tend to ignore harlequin shrimps, no study has ever determined whether or not these shrimps are toxic or unpalatable to fish. Eyespots present in some decapod species are known to be used as threatening visual cues in intraspecific interactions (Bedini *et al*., 2002). However, it remains to be investigated as to whether the ocelli present in some coral reef shrimps (e.g. *Periclimenes brevicarpalis* and *Saron* spp.) also play an antipredatory role similar to that of ocelli in insect wings (Stevens, 2005).

3.2 Sea anemone–shrimp associations

In the complex habitats of coral reefs, several shrimp species have established more or less stable relations in, on or with other macro-invertebrates and several fishes (Bruce, 1976a, b). In all these associations there is a trade-off between the costs of leaving the host and the benefits of staying (Thiel & Baeza, 2001). When a species is closely associated with other organism(s), it usually benefits from additional protection and food availability. However, these associations may also present substantial costs, namely the need to actively defend the host species and also a restriction in the species' mobility (when the shrimp is associated with sessile organisms or occupies a specific territory) (Baeza & Thiel, 2003). The 'price to pay' for these associations is more pronounced when the host species cannot entirely fulfil all the needs of the shrimp and it has to venture away to search for additional food or mating partners (Thiel *et al*., 2003). Nevertheless, many shrimp species associate with their hosts in groups of individuals of both sexes or in heterosexual pairs (Knowlton, 1980; Nizinski, 1989; Omori *et al*., 1994).

Sometimes, shrimps that normally do not associate with sea anemones may seek protection from predators from these organisms, namely when threatened in their vicinity (Calado *et al*., 2007a). A classical example of such associations is that of partner shrimps *Periclimenes pedersoni* and *P. yucatanicus*, as well as sexy shrimps *Thor amboinensis*, with large sea anemones. These species associate in large numbers in the same host, and do not display agonistic behavior towards conspecifics (Mahnken, 1972; Wirtz, 1997). These

groups harbour both male and female shrimps, and the occurrence of ovigerous and juvenile shrimps is not unusual in the same host sea anemone. Curiously, the true nature of these associations between shrimps and sea anemone, commonly labelled as mutualism, is still not clear. Fautin *et al.* (1995) showed that *Periclimenes brevicarpalis* clips and feeds on the tentacles of its host anemone without any evident benefit for the host. This type of interaction is certainly closer to parasitism, if this behavior negatively affects the host, or to commensalism, if it has no effects on the host. If, as recorded by Spotte (1996), host anemones with zooxanthellae benefit from ammonia produced by symbiotic shrimp living among their tentacles, then the association between shrimps and sea anemones may truly be designated as mutualism.

Goy (1990) has demonstrated the need for a chemical cue from host sea anemones to induce the settlement of larval *Periclimenes*. However, Sarver (1979) and Dos Santos *et al.* (2004) have also shown that *Thor* and *Periclimenes* larvae, respectively, are not immune to the stinging cells of the host anemone, with even brief contacts proving to be lethal for the larvae. In this way, it is therefore legitimate to assume that partner shrimps, and probably also other shrimps associating with sea anemones, settle near their host species and only later will they seek shelter in sea anemones. Sarver (1979) points out that host anemones that commonly stung and ate late-stage sexy shrimp larvae were never recorded preying post-metamorphic shrimps. How shrimp species acquire immunity to the anemone's stinging cells is still unknown. The degree of immunity acquired by each shrimp species may also differ, since symbiotic shrimps do not display the same spatial distribution in their host (Khan *et al.*, 2004). As an example, the sexy shrimp *T. amboinensis* is commonly recorded near the mouth of sea anemones, an area readily avoided by most *Periclimenes* species. The fidelity of shrimp species to their host sea anemone is variable and species specific. Shrimp species associating with a single host sea anemone are generally termed specialists, while those that may associate with a wide group of hosts, for example *T. amboinensis* and *P. brevicarpalis*, are termed generalists (Guo *et al.*, 1996; Khan *et al.*, 2003). This particular aspect of ornamental shrimp biology is of great interest for the aquarium trade since it allows traders to select those species that display a wider range of potential hosts and that therefore have a higher probability of being successfully kept in captivity.

3.3 Fish cleaner shrimps

From all interspecific associations already recorded involving shrimps, cleaning symbioses are certainly some of the most fascinating (see color plate 9). These associations commonly involve a cleaner (either a fish or a shrimp) and a usually larger 'client' (commonly a fish) and can be divided into two categories: incidental, where neither the cleaner nor the client has developed special adaptations for their roles; and true cleaning behavior, where both

cleaner and client display specific behaviors and morphology that facilitate cleaning (Côté, 2000). Although cleaning symbioses are very well known from coral reefs, they have also been recorded in subtropical and warm temperate waters (Van Tassel *et al*., 1994; Wirtz, 1995; Wirtz & Debelius, 2003). Nevertheless, despite the phylogenetic distances of cleaner and client species, as well as the diverse habitats in which these associations have already been recorded, marine cleaning associations exhibit a similar macroecological pattern worldwide (Floeter *et al*., 2007). Shrimp species already recorded cleaning fishes are listed in Table 3.1, with emphasis on the ones commonly available in the aquarium trade.

Cleaner shrimps are commonly believed to remove ectoparasites, bacteria, dead pieces of body tissue or other particles from the buccal cavity, gills and body surface of their fish clients. The existence of cleaning symbiosis involving shrimps has been known for many years, and early works have contributed to the general idea that cleaners play a crucial role in the health of their fish clients as well as in their distribution patterns (Limbaugh, 1961; Limbaugh *et al*., 1961). This perception of cleaning symbiosis made them a classical textbook example of mutualism (Jonasson, 1987; Thompson, 1994). However, Spotte (1998) pointed to evidence that 'cleaning symbioses' are only inferential, being based entirely on the observation of associations (e.g. Wicksten, 1998; Kulbicki & Arnal, 1999) and noted that shrimps actively removing and/or ingesting fish parasites have never been recorded. Sargent & Wagenbach (1975) reported that tactile stimulation provided by the cleaner shrimps may actually be the only 'reward' for fish clients.

Despite the laboratory experiments of Bunkley-Williams & Williams (1998), verifying the ability of *P. pedersoni* to remove juvenile parasitic cymothoid isopods from fishes, only recently have Becker & Grutter (2004) finally provided irrefutable scientific evidence that 'cleaner shrimps do clean'. The authors verified that *Urocaridella* sp. c and *P. holthuisi* removed and ingested fish ectoparasites, with the latter species being able to reduce the load of monogenean ectoparasites from fish clients by 74.5% in only 48 hours (but under laboratory conditions). The gut content of specimens from both species collected from the wild demonstrated that they do ingest crustacean ectoparasites. These results differ from those recorded by Turnbull (1981) for *P. pedersoni*, since the gut content analysis of this popular cleaner shrimp only revealed the presence of fish mucus and detritus. According to Becker & Grutter (2004), the two species of shrimps addressed in their study may display different dietary preferences, since *Urocaridella* sp. c displayed a higher number of ectoparasites in the diet. The authors suggest that this species may be somehow more specialized to remove crustacean ectoparasites from fishes. However, no obvious morphological adaptation seems to be present in the members of genus *Urocaridella* that indicates any degree of specialization for ectoparasite removal. Becker & Grutter (2005) have also verified that *Urocaridella* spp positively discriminate parasitized over unparasitized fishes and that starved cleaner

Table 3.1 Aquarium requirements, associative behavior, stocking limitations and suitability of the most commonly traded marine ornamental shrimp species

Family	Species	Special aquarium requirements	Stocking limitations	Associative behavior	Reef safe
Stenopodidae	*Stenopus cyanoscelis*	Small rock cave	One mated pair per aquarium[3]	—	Yes
	Stenopus hispidus	Medium-sized/large rock cave	One mated pair per aquarium[3]	Fish cleaner	Yes[7]
	Stenopus pyrsonotus	Medium-sized/large rock cave	One mated pair per aquarium[3]	Fish cleaner	Yes[7]
	Stenopus scutellatus	Small rock cave	One mated pair per aquarium[3]	—	Yes
	Stenopus spinosus	Medium-sized rock cave	One mated pair per aquarium[3]	—	Yes
	Stenopus tenuirostris	Small rock cave	One mated pair per aquarium[3]	—	Yes
	Stenopus zanzibaricus	Small rock cave	One mated pair per aquarium[3]	—	Yes
Alpheidae	*Alpheus ochrostriatus*	Deep sand bed[1]	One mated pair per aquarium[3]	With gobies	Yes
	Alpheus randalli	Deep sand bed[1]	One mated pair per aquarium[3]	With gobies	Yes
Gnathophyllidae	*Gnathophyllum americanum*	Customized small aquarium	Several specimens may be stocked	—	Yes
Hippolytidae	*Lysmata amboinensis*	Small/medium-sized rock cave	One mated pair per aquarium[4]	Fish cleaner	Yes
	Lysmata ankeri	Small rock cave	Large groups may be stocked	—	Yes
	Lysmata boggessi	Small rock cave	Large groups may be stocked	—	Yes
	Lysmata californica	Small rock cave	Large groups may be stocked	Occasional fish cleaner	Yes

Continued

Table 3.1 (Continued)

Family	Species	Special aquarium requirements	Stocking limitations	Associative behavior	Reef safe
Hippolytidae (Continued)					
	Lysmata debelius	Small/medium-sized rock cave	One mated pair per aquarium[3]	—	Yes
	Lysmata grabhami	Small/medium-sized rock cave	One mated pair per aquarium[5]	Fish cleaner; occasionally with sea anemones	Yes
	Lysmata kuekenthali	Small rock cave	Large groups may be stocked	—	Yes
	Lysmata rathbunae	Small rock cave	Large groups may be stocked	—	Yes
	Lysmata seticaudata	Small rock cave	Large groups may be stocked	Occasional fish cleaner	Yes
	Lysmata vittata	Small rock cave	Large groups may be stocked	—	Yes
	Lysmata wurdemanni	Small rock cave	Large groups may be stocked	—	Yes[8]
	Lysmatella prima	Small rock cave	Large groups may be stocked	—	Yes
	Parhippolyte mistica	Medium-sized/large rock cave	Several specimens may be stocked	—	Yes
	Saron marmoratus	Small rock cave	Large groups may be stocked[6]	—	No[9]
	Saron neglectus	Small rock cave	Large groups may be stocked[6]	—	No[9]
	Saron rectirostris	Small rock cave	Large groups may be stocked[6]	—	No[9]
	Thor amboinensis	Customized small aquarium with a large sea anemone	Large groups may be stocked	With several large sized sea anemones	Yes

Hymenoceridae	*Hymenocera elegans*	Small rock cave; require starfish[2]	One mated pair per aquarium[3]	—	Yes[10]
	Hymenocera picta	Small rock cave; require starfish[2]	One mated pair per aquarium[3]	—	Yes[10]
Palaemonidae	*Periclimenes brevicarpalis*	Host anemone	Several specimens may be stocked	With sea anemones	Yes
	Periclimenes pedersoni	Host anemone	Large groups may be stocked	Fish cleaner; with sea anemones	Yes
	Periclimenes yucatanicus	Host anemone	Large groups may be stocked	With sea anemones and corallimorphs	Yes
	Periclimenes holthuisi	Host anemone	Large groups may be stocked	With sea anemones and large polyp corals	Yes
	Urocaridella antonbruunii	—	Large groups may be stocked	Fish cleaner	Yes
Rhynchocinetidae	*Rhynchocinetes durbanensis*	Small rock cave	Large groups may be stocked[6]	—	No[9]

[1] The excavation activity of these shrimps may sometimes cause live rocks to move and eventually fall if these are not firmly placed in the aquascape of the aquarium.
[2] These shrimps will only survive in captivity if live starfishes are regularly added to the aquarium, since these are the only dietary items of their feeding regime.
[3] These shrimps will readily attack conspecifics of the same sex and sometimes agonistic behaviors may also be displayed towards conspecifics of the opposite sex.
[4] Unless if stocked in an aquarium of more than 500 litres. Occasionally, more than two specimens may tolerate each other in smaller aquariums, particularly when stocking small male-phase specimens.
[5] In the wild it is not unusual to record trios of ovigerous shrimps (Wirtz & Debelius, 2003).
[6] Avoid stocking dominant males together (shrimps exhibiting the first pair of chelipeds greatly enlarged).
[7] Anecdotal observations note that large specimens in captivity may capture small fishes.
[8] Anecdotal observations note that members of the *wurdemanni* species complex may attack soft corals in captivity.
[9] These shrimps will nip the mantle of tridacnid clams, as well as damage corals, snails, feather duster worms and other invertebrates in the aquarium.
[10] These shrimps will obviously be a threat to starfishes.

shrimps cleaned parasitized fishes more often than when satiated. In this way, higher hunger levels significantly increase a cleaner shrimp's willingness to clean fishes, even though it risks being eaten by its client. The work by Becker & Grutter (2005) highlights how fish cleaning by shrimps also concurs with optimal foraging theory, which suggests that a forager should choose the patch with the greatest amount of food to optimize its energy gain (Stephens & Krebs, 1986).

Jonasson (1987) described the following list of qualitatively stereotypical behaviors displayed by several cleaner shrimps in the genera *Stenopus*, *Lysmata* and *Periclimenes* when interacting with fish clients:

(1) shrimp orients to fish client;
(2) shrimp's antenna taps the fish;
(3) the shrimp approaches the fish;
(4) the shrimp climbs on to the body surface of the fish;
(5) the shrimp cleans the fish's ventral area; and
(6) the shrimp cleans the fish's dorsal area.

According to Wicksten (1998), touching the client fish with their antennae before cleaning may aid in the recognition of a client. It is also suggested that this action may provide an input on the nutritional value of the fish's mucus, since shrimps have chemosensory setae on their antennae. Kishida *et al.* (1992) suggested that the vitellogenin present in fish mucus might have pheromonal functions, which could trigger cleaning behavior in inspecting shrimps.

Becker *et al.* (2005) have provided detailed information concerning the rocking dance used by *Urocaridella* to advertise its cleaning services to potential fish clients. The shrimp uses a stereotypical side-to-side movement, or rocking dance, while approaching the client fish and the dance is always followed by cleaning. It is also worth noticing that hungry cleaners spend more time rocking and that parasitized fishes prefer them to satiated shrimps. The vigorous whipping of conspicuous white antennae and lateral body swaying while standing has also been described as an 'advertising dance' performed by other cleaner shrimps (e.g. *Stenopus* and *Periclimenes*) (Limbaugh *et al*., 1961). As suggested for cleaner fishes (Stummer *et al*., 2004), it appears that there may exist a convergent evolution of cleaner-shrimp signalling, which facilitates their recognition by fishes.

Regardless of shrimps effectively 'advertising' their cleaning services, it is pertinent to ask: what prevents a fish from eating a cleaner shrimp? Grutter (2004) suggested that cleaner fishes use tactile stimulation as a preconflict management strategy. It is possible that cleaner shrimps use a similar tactic, namely gently touching their client fishes with the antennae or pereiopods. According to Trivers' explanation for reciprocal altruism (Trivers, 1971), it appears that a fish will refrain from eating a cleaner shrimp when repeated

removal of parasites by the cleaner is of greater benefit to the fish than eating the cleaner. However, it is known that cleaner shrimps are exposed to predation by some of their fish clients. It is still not clear why: (1) fishes with reduced parasite loads, after being cleaned, refrain from eating the shrimp, since their needs for cleaning services have also decreased; (2) when hunger levels increase in a client fish, it does not consume the cleaner shrimp since this would be beneficial in the short term. The question raised by Poulin & Vickery (1995) on the evolutionary game of cleaning symbiosis, i.e. 'To cheat or not to cheat?', therefore remains open for cleaner shrimp associations with fishes.

Limbaugh (1961) popularized the term 'cleaning stations' to describe the places where cleaning organisms advertise their services to potential fish clients. These areas are generally located in coral heads, depressions on rocky bottoms and shipwrecks. The long white antennae exhibited by some popular cleaner shrimps (e.g. *Stenopus hispidus*, *Lysmata amboinensis* and *L. grabhami*) at cleaning stations are commonly considered as a special feature used to attract potential fish clients. If this was the case, how could shrimps with smaller white antennae (e.g. *Periclimenes*) advertise their services? It could be argued that *Periclimenes* species usually associate with large sea anemones, which are highly conspicuous and could easily be found by parasitized fish seeking the services of cleaner shrimps. Wicksten (1998) recorded that *Periclimenes pedersoni* inhabiting the giant anemone *Condylactis gigantea* (Weinland, 1860) was never recorded more than 10 cm away from their tentacles, thus their cleaning stations were confined to the immediate vicinity of the host sea anemone. The question arises as to how cleaner shrimps in the genus *Urocaridella* can be such effective fish cleaners if they do not present either long or white antennae and do not associate with large and conspicuous invertebrates such as sea anemones. Another pertinent question is why certain small fish species approach cleaner shrimps located near lethal sea anemones (e.g. *Lysmata grabhami* associates with the club-tipped anemone *Telmatactis cricoides* (Duchassaing, 1850) (Wirtz, 1997)) and moray eel burrows. Will the benefit of having ectoparasites removed compensate for the risk of being predated? The question 'What makes a shrimp a cleaner shrimp?' is still far from being properly answered.

3.4 Goby–shrimp associations

Alpheid shrimps are well known for their snapping ability, which in some cases can be so powerful that it has led to their being given the popular name of pistol shrimps. The studies by Versluis *et al.* (2000) revealed that the snap results from implosion of a cavitation bubble caused by the rapid ejection of water from a socket in the fixed finger by a plunger (specialized tooth) on the

dactylus. In fact, the dactylus closure is among the most rapid movements in the animal kingdom (Schmitz, 2001).

However, the feature that makes these shrimps highly demanded in the aquarium trade is their associative behavior with gobiid fishes. Some alpheid species exhibit facultative associations with gobiids, while others appear to be obligate symbionts (Karplus, 1992). In both situations, shrimps and gobies share the same burrow, and communication at interspecific level must take place between the organisms. The gobiid fish uses the burrow excavated by alpheids, though not exclusively (Farrow, 1971), the shrimps removing impressive amounts of sand and coral rubble with the help of the chelipeds, namely the large snapping claw (Luther, 1958; Magnus, 1967). While the burrow only acts as a temporary shelter during the day, it is a permanent refuge for both organisms during the night. Alpheid shrimps' burrows are irregular and in close contact with hard objects within the substrate. The study performed by Karplus *et al.* (1974) on the architecture of nearly 500 burrows occupied by sympatric goby–shrimp associations revealed that their upper parts, the number of burrow openings and the structure of the burrows exhibit species-specific features. As an example, alpheid shrimps may present tunnel-like or funnel-shaped burrow entrances, and while most burrows have a single opening, some species build up to three different openings. Burrows with approximately 3 cm diameter are usually 80–120 cm long, reaching a depth around 30–40 cm. A single burrow occupied by *Alpheus djiboutensis* De Man, 1909 was 4 cm wide, 220 cm long and 55 cm in depth (Karplus *et al.*, 1974). These architectural features may never reach such dimensions in reef aquariums, since hobbyists only use 5 to 10 cm of coral reef sand in the bottom of their tanks. As a consequence of the shrimp's feeding activity, which permanently removes substrate from the area above the burrow, there is a continuous backward shift in the burrow's opening (Magnus, 1967).

The near blindness, or at least reduced vision, of alpheid shrimps means that they strongly rely on the watching capacities of their gobiid partners as an alarm system against predation (Karplus *et al.*, 1972a). In this way, a shrimp only emerges from the burrow in the presence of the goby and maintains its antennae in contact with the fish (Yanagisawa, 1984; Karplus, 1987). A simple tail-quivering motion by the goby, or simply a sudden movement, triggers the shrimp's retreat into the burrow (Preston, 1978). Karplus *et al.* (1972b) analysed the mutual attraction of goby–shrimp associations and concluded that, whereas the fish is attracted by its partner visually, the shrimp appears to be chemically attracted. The same study confirmed that this mutual attraction seems to be reinforced by the existence of negative phototaxis and positive thigmotaxis of both organisms, which attracts them to a common burrow.

After surveying a significant number of burrows harbouring *Alpheus bellulus* Miya & Miyake and *Amblyeleotris japonica* Takagi, 1957, Yanagisawa (1984) predicted that it must be essential for both species to associate as soon as possible after settlement, in order to increase their chances of escaping predators. After

verifying that certain burrows with a juvenile shrimp were still not occupied by a partner fish, the same author hypothesized that perhaps alpheid shrimps start digging a small burrow immediately after settling. After this event, a free-swimming or benthic stage gobiid exploring the bottom will eventually enter the burrow and associate with the shrimp. Although Yanagisawa (1984) suggested that gobies and alpheid shrimps remain together for relatively long periods, Karplus *et al.* (1974) reported that large *Cryptocentrus caeruleopunctatus* (Rüppell, 1830) were able to dislodge smaller sized gobies from their burrows and that after only a few minutes the 'invading' goby and the alpheid shrimp had already associated, assuming their normal activities in the burrow's entrance.

Several alpheid shrimps show well developed orbital hoods, since these morphological features may provide valuable protection to the shrimp's eyes from its excavating activity and its own powerful snap. Interestingly, in experiments with *Alpheus heterochaelis*, a species with a powerful snapping claw, Schmitz & Herberholz (1998) did not observe injuries among antagonists because intraspecific fights are largely ritualized. However, interspecific encounters often result in serious injuries, including loss of chelipeds or other appendages, and possibly also in eye injuries (Anker *et al.*, 2006).

3.5 Other remarkable associations

Besides associating with gobies, alpheid shrimps are also known to associate with other crustaceans, such as crabs (Silliman *et al.*, 2003) and stomatopods (Froglia & Atkinson, 1998). An interesting association, which can be easily displayed in a reef aquarium, is that of the alpheid shrimp *Alpheus lottini* Guérin-Méneville, 1829, and the trapezid crab *Trapezia ferruginea* Latreille, 1828, inhabiting corals of the genus *Pocillopora* Lamarck, 1816. The record by Lassig (1977) on these two organisms in the field highlighted how they cooperated to defend their host from potential predators. Lassig (1977) assumed that the physical interactions between them were probably limited to the cleaning activities of the shrimp in relation to the crab. In a later study, Vannini (1985) verified that, in order to avoid being attacked by trapezid crabs when trying to enter the host coral, alpheid shrimps performed certain appeasement movements, which appeared to be part of the crab's appeasement repertoire. In this way, the weaker shrimp had to learn to 'speak crab-ese' to prevent aggression by the stronger trapezid crab also inhabiting their host coral.

Sometimes, certain shrimp species associate with organisms that commonly include similar species in their diets. *Periclimenes magnificus* Bruce, 1979 is commonly recorded in association with the snake eel *Callechelys* Kaup, 1856, with shrimps wandering dangerously close to the mouth of this fish predator (Debelius, 2001). Why the eel tolerates the shrimp is still a mystery.

Another puzzling association is that of stenopodidean and caridean shrimps with stomatopods, also known as mantis shrimps. Mantis shrimps are among some of the most successful and voracious crustacean predators in the sea, being able to unleash lethal smashing or spearing attacks at lightning speed (as fast as 4 milliseconds) (Burrows, 1969; Caldwell & Dingle, 1975). This fearsome reputation makes them highly undesirable in reef aquariums, although they are many times involuntarily introduced in these displays as hitchhikers in live rock. *Stenopus tenuirostris*, *P. imperator* and *T. amboinenis* have all been observed in association with *Lysiosquillina lisa* Ahyong & Randall, 2001, with some of these shrimps even being recorded either over or between the eyes of the stomatopod (Debelius, 2001). It seems unlikely that the stomatopod truly needs the services of these potential cleaners, since mantis shrimp are able to reach and clean any portion of their body. Stenopodidean and caridean shrimps may be attracted by the mucus commonly employed by stomatopods to build their burrows and/or by their leftovers and regurgitated material (Debelius, 2001). Currently there is still no scientific explanation for these unlikely associations and why these bold shrimps are not predated by stomatopods.

Some of these remarkable associations may also be observed in captivity, as long as these specimens are properly stocked in captivity (see Table 3.1 for special stocking requirements of marine ornamental shrimps).

3.6 *Hymenocera* and their starfish diet

In general, shrimps feed on a large variety of prey and do not display particularly selective feeding regimes. However, shrimps in the genus *Hymenocera* have a feeding regime that sometimes poses problems to hobbyists wishing to display them in reef aquariums: they feed exclusively on starfish (see color plate 5) (Wickler & Seibt, 1970). Either in the field or in aquaria, harlequin shrimp largely prefer starfishes with arms that are triangular in section, namely those in the family Ophidiasteridae Verrill, 1870 (*Linckia* Nardo, 1834, *Leiaster* Peters, 1852 and *Nardoa* Schmidt, 1862). These shrimps are also known to accept the starfishes *Oreaster* Müller and Troschel, 1842, *Ceramaster* Verrill, 1899, *Protoreaster* Döderlein, 1916, and *Pentaceraster* Döderlein, 1916, as food (Wickler, 1973; Seibt & Wickler, 1979), as well as the eastern Atlantic starfishes (non-natural prey) *Marthasterias* Jullien, 1879 and *Asterina* Nardo, 1843 (Calado, unpubl. obs.). Seibt & Wickler (1979) reported that, when collecting *Hymenocera* in the reefs, certain areas were occupied by hundreds of autotomized starfish arms, generally 2–3 cm long. This abundance of potential prey means that most probably harlequin shrimp have very limited food competition in their natural habitats. Harlequin shrimps localize their prey through chemical cues (Wickler & Seibt, 1970; Rainbow, 1974; Wasserthal & Seibt, 1976), and

certainly benefit from their highly developed sense of smell (Seibt, 1973, 1974). The shrimps commonly flip over their prey and devour it alive, sometimes over several days. Shrimps seem to ingest the ambulacral feet, as well as intestinal, nervous and gonad tissues, and have been recorded feeding from the tip of the arm towards the central disc of starfish (Debelius, 2001). This feeding strategy probably allows *Hymenocera* to keep their prey alive for longer periods.

Despite living in pairs, harlequin shrimps do not seem to cooperate while foraging, not even when both members of the pair converge towards the same prey (Seibt & Wickler, 1979). Male and female harlequin shrimp seem equally efficient at hunting starfish, although males seem to be faster at finding new prey. Curiously, conspecifics only tolerate each other (including members of a mated pair) when feeding on large prey and at distances greater than 5 cm (Seibt & Wickler, 1979).

Hymenocera are also able to effectively capture and kill the famous crown of thorns starfish *Acanthaster planci* (Linnaeus, 1758), a well known coral predator. This particular organism has also contributed to the endangered status of certain coral reef areas (De'ath & Moran, 1998a, b; Pratchett, 2005). Despite the initial enthusiasm about the potential use of harlequin shrimps as a biological weapon to fight the crown of thorns starfish (Talbot & Talbot, 1971), it seems that only starved harlequin shrimp with no other prey available will attack *Acanthaster* (Wickler, 1973). Therefore, it is unlikely that *Hymenocera* will prey upon the crown of thorns in a coral reef where linckiid starfishes, the shrimp's favourite prey, are also present.

Chapter 4

Reproductive Biology and Mating Behavior

The mating behavior of decapod crustaceans has been widely studied in recent decades, in particular the mechanisms involved in sexual selection (Correa & Thiel, 2003). These studies are of major relevance for researchers addressing evolutionary ecology, but also for those willing to successfully keep breeding pairs of decapods in captivity and wishing to maximize their reproductive output. While the majority of decapod species displays separate sexes, certain shrimp species may change sex during their lifetime and several different patterns have already been described (e.g. Bauer, 2000). Because of their phylogenetic distance, the reproductive biology and mating behavior of stenopodidean and caridean shrimps will be addressed separately in this chapter.

4.1 Stenopodidean shrimps

Shrimps from the genus *Stenopus* display separate sexes and are known to occur in heterosexual pairs. This particular aspect of their sociobiology has always fascinated researchers. The first work reporting pair formation in the genus was that of Brooks & Herrick (1893) for *S. hispidus*. Because of its large distribution area, as well as its commercial importance, the majority of scientific works available on stenopodid reproductive biology are biased towards *Stenopus hispidus*. Only several years after Brooks & Herrick's (1893) work have several pertinent questions started to be addressed, namely:

(1) How readily do these shrimps form pairs?
(2) Are wild shrimp pairs always heterosexual?
(3) How is intraspecific sex recognition accomplished?

Johnson (1966, 1969) confirmed that in the wild *S. hispidus* readily forms pairs and that these pairs are always heterosexual. The author also recorded that *Stenopus* can detect each other by visual, olfactory and/or tactile means. The olfactory sense strengthens the rise in courtship and agonistic motivation,

while visual contact inhibits that motivational rise. Johnson (1966, 1969) also concluded that tactile contact decreases agonistic motivation, possibly due to sex recognition. In this way, *Stenopus* initially orientate themselves towards another shrimp by visual and chemical detection. As a result of the unspecific nature of these factors an agonistic response is triggered. Posterior tactile contact allows sex recognition, decreases agonistic motivation and increases the motivation towards courtship behavior. Additionally, Johnson (1969) also states that open chelipeds and raised bodies are fixed motor patterns for threatening or attacking specimens, whereas closed chelipeds and crouched bodies (walking legs are flexed in such a way that the shrimp's body is closer to the substrate than usual) are motor patterns associated with appeasement behavior.

Johnson (1977) recorded that individual *Stenopus* behave differently when re-paired with previous pair members or individuals with which they had no previous interactions. This evidence supports the existence of individual recognition in *S. hispidus*, with shrimps being able to recognize previous mates even when separated from them for up to 6 days. Failure to recognize previous mates after longer separation periods may be regarded as indirect evidence that *Stenopus* pairs rarely separate in the wild. Additionally, the ability to retain individual recognition for longer periods may negatively affect the survival of the species, since it would inhibit the formation of new pair bonds after a member of a pair naturally loses its mate (e.g. due to predation) (Johnson, 1977).

Large *Stenopus* females are highly aggressive towards smaller males and easily dominate them (Johnson,1969). This behavior agrees with the general patterns described by Reese (1964) for decapod behavior, with larger specimens usually dominating smaller ones. However, this does not prevent pair formation between *Stenopus* with large size differences (Yaldwyn, 1966).

Zhang *et al*. (1998a) have provided a detailed description of *S. hispidus* mating behavior, highlighting five different steps.

(1) Antennule contact: shrimps establish antennule contact and gently wave their large third pair of chelipeds at each other. This step lasts from 10 minutes to 6 hours.
(2) Erection of the female body: female shrimp generally turns around with the abdomen raised.
(3) Grasping: male rapidly approaches the female, also displaying its abdomen in a raised position, and holds the female, abdomen to abdomen.
(4) Mating: the male turns rapidly (150–180°) to face the female abdomen to abdomen and head to tail, with copulation occurring next and lasting approximately 10 seconds.
(5) Spawning: after mating, the male leaves the female, with spawning taking place in the next 15 to 25 minutes and lasting about 10 minutes.

Like most decapod crustaceans, female *Stenopus* are also only receptive to mating after moulting. Johnson (1969) described the 'mating dance' exhibited by *Stenopus* as a fixed motor pattern associated with courtship behavior.

Female *S. hispidu*s are thought to reach sexual maturity at a total length of 30 mm (Stolen, 1964) and, like several other shrimp species (Corey & Reid, 1991), display a significant relationship between body size and the number of embryos produced (Chockley & St. Mary, 2003). As an example, a female with a total length of approximately 4.5 cm may incubate more than 2500 embryos (Chockley & St. Mary, 2003).

Tropical species of *Stenopus*, under stable abiotic conditions, are continual breeders (see Bauer (1989) for further details). Females incubating embryos in the abdomen display developing ovaries. When the embryos are about to hatch (displaying a silvery coloration and well developed eyes), large vitellogenic oocytes are clearly visible in the ovaries located in the dorsal portion of the female's carapace. Soon after larval hatching, the female moults, mates and spawns, initiating a new cycle of ovarian maturation and embryo incubation.

4.2 Caridean shrimps

Marine ornamental caridean shrimps may either be gonochoric (display separate sexes) or protandric hermaphrodites (specimens will first mature as males and later change to functional females). In the absence of well defined sexual dimorphism, certain morphological features may be more or less easily used to sex shrimps, namely the positioning of the genital openings or gonopores (Figure 4.1).

Caridean shrimps in the same family, or even in the same genus, may display different sexual systems, making generalizations on this subject misleading (Correa & Thiel, 2003). None the less, there are certain shrimp families that seem rather conservative concerning their sexual systems. All Palaemonidae, Hymenoceridae and Rhynchocinetidae shrimp species, commonly available in the aquarium industry, display separate sexes, with more or less pronounced sexual dimorphism. Although alpheid shrimps may either be gonochoric or protandric hermaphrodites with primary males (shrimps born as males will later change sex to female, while other shrimps remain as males through their entire lifespan), members of the genus *Alpheus* apparently exhibit separate sexes (Knowlton, 1980). Currently, there is no available information concerning the sexual system of gnathophyllid shrimps.

Hippolytid species can be gonochoric or display several variations of protandric hermaphroditism. In the hippolytid genus *Thor*, some species are gonochoric whereas others are partial protandric hermaphrodites with primary males and females (there are shrimps changing sex from male to female, but also permanent males and females) (Bauer & VanHoy, 1996).

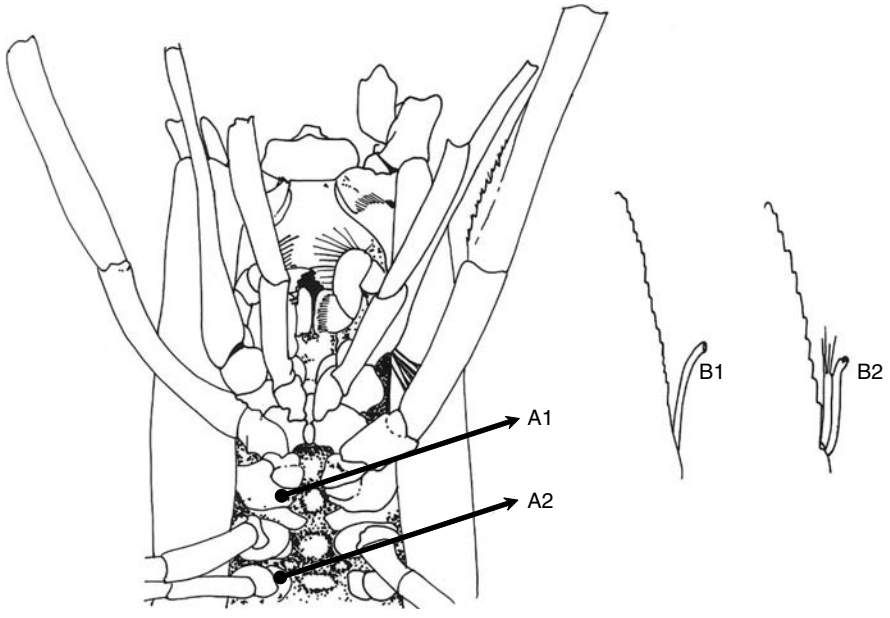


Figure 4.1 Diagnosing sexual features of caridean shrimps: A1, female gonopore (or genital opening) located in the coxa of the third pair of pereiopods; A2, male gonopore (or genital opening) located in the coxa of the fifth pair of pereiopods; B1, detail of the endopod of the second pair of pleopods of a female shrimp displaying an appendix interna; B2, detail of the endopod of the second pair of pleopods of a male shrimp displaying an appendix interna and an appendix masculinae. Drawings are not to scale.

There is no available information on the sexual system of the sexy shrimp *Thor amboinensis*. The existing studies on the biology of *Saron* do not discuss their sexual system in detail (Kruschwitz, 1967; Tirmizi & Kazmi, 1971). However, because of the pronounced sexual dimorphism exhibited by *Saron*, it is likely that marble shrimps display separate sexes. Concerning the genera *Parhippolyte* and *Lysmatella*, there is no available information addressing their sexual systems.

The genus *Lysmata* displays a puzzling, recently described and so far unique sexual system among decapod crustaceans: protandric simultaneous hermaphroditism (shrimps first reproduce as males and then become simultaneous hermaphrodites, but are not able to self-fertilize) (Bauer, 2000). The presence of testicular portions and male ducts in the so-called female phase of *Lysmata seticaudata* was early noticed by Spitschakoff (1912). Curiously, despite the remarkable quality of research addressing the endocrinology of sex reversal in crustaceans, using *Lysmata seticaudata* as a model, the occurrence of simultaneous hermaphroditism has never been discussed (Charniaux-Cotton, 1958, 1959a, b, 1985; Charniaux-Cotton & Touir, 1973; Touir, 1977). Subsequent anecdotal reports of aquarium hobbyists describing the production of viable embryos by both members of *Lysmata* pairs led researchers to re-examine the

sexual system of these shrimp. Bundy (1983) and Crompton (1992) confirmed this ability, with the first author even providing some histological evidence on the occurrence of mature oocytes and spermatocytes in the same gonad of '*Lysmata wurdemanni*' (currently, because of the description of the *wurdemanni* species complex by Rhyne & Lin (2006) it is not possible to be sure of the species analysed). However, this research did not rule out the possibility of self-fertilization or exogenous sperm storage since the authors were unable to accurately demonstrate functionality, a major aspect for the determination of hermaphroditism (Sadovy & Shapiro, 1987). Evidence for the existence of functional simultaneous hermaphroditism in the genus *Lysmata* was finally provided by Bauer & Holt (1998) and Fiedler (1998). Currently, with the growing evidence for protandric simultaneous hermaphroditism in many other *Lysmata* species (e.g. Udekem d'Acoz, 2003; Bauer & Newman, 2004; Rhyne & Lin, 2006), it is widely accepted that all species in the genus display this sexual system.

Caridean shrimps commonly available in the aquarium trade are continuous breeders, with females carrying developing embryos in the abdomen while their ovaries continue to mature. In this way, after larval hatching the female will moult, be receptive to males, mate, spawn and initiate a new embryo incubation cycle.

The study by Bauer (1976) on *Heptacarpus pictus* (Stimpson, 1871) is still one of the most detailed descriptions of the mating behavior displayed by caridean shrimps and will be used to provide a general overview on this subject, although species-specific variations are known to occur (e.g. Nouvel, 1939; Correa *et al.*, 2000). Commonly in caridean shrimps precopulatory behavior is absent. The mating event is actually a sequence of stereotyped actions.

(1) Contact: males only detect receptive females (those with mature ovaries, which have recently moulted) after touching the female, usually with their antennal flagellum (contact chemoreception). Carlisle (1962) refers to the apparent lack of a distance pheromone in caridean shrimps and believes that probably a non-diffusible substance in sea water covering the surface of the female's exoskeleton is the sex pheromone. However, Zhang & Lin (2006) have shown the existence of a distance pheromone involved in mate recognition in *Lysmata wurdemanni*, with that substance only being detected by receptors on the outer flagella of the antennules. Nevertheless, the same authors also point out that contact pheromone may be more important than distance pheromone in the mating process. Upon detecting a receptive female, the male's behavior changes dramatically and it starts trying to seize the female with its pereiopods.
(2) Climb: after contact, the male tries to crawl up to the dorsal midsection of the female.

(3) Straddle: while clutching the female with his pereiopods, the male is astride the female's dorsal midline with the anterior ends of both shrimps facing the same side. Apparently, this action allows the female to settle and accept the presence of the male. After reaching this position, male shrimps can usually successfully mate. The female's rejection usually occurs in the contact and climb phases.
(4) Mount: the male swings the body to both sides of the female, positioning its abdomen along the female's anterior abdominal region.
(5) Dip: the male swings under the female and positions its thoraco-abdominal junction beneath and perpendicularly to the female's first abdominal sternite.
(6) Pleopod beat: the male briefly beats the pleopods in the dip position described above and at this time the spermatophore is emitted and transferred. The male shrimp deposits the spermatophore on the underside of the female's first abdominal segment. These sperm packets are extruded from the male's genital openings (in the bases of the fifth pair of pereiopods), and are composed of sperm mixed in a mucoid material. The spermatophores adhere to the female's smooth abdominal sternite. The largely expanded abdominal pleurae of females prevent the male's genital opening from contacting the female's abdominal sternite. In this way, the successful transfer of the spermatophore from the male to the female results from the action of the modified endopods of the first two pairs of pleopods during copulation.
(7) Disengagement: male and female separate just after the pleopod beat.

After copulation, females commonly clean their posterior thoracic sterna, the bases of the walking legs, and especially the abdominal sterna and pleopods with the help of their second chelipeds. Since females have recently moulted, pre-spawning grooming is probably more related to arrangement of setae involved in spawning than in removing particulate debris (Höglund, 1943). The cleaning behavior performed by females breaks and spreads the spermatophore about the area where it is deposited on the anterior surfaces of the endopods of the first pair of pleopods. Newly extruded eggs must pass between the endopods and abdominal sternite during spawning in order to ensure fertilization. Egg extrusion may occur briefly after copulation or it may take up to 24 hours (Bauer, 1976). In the absence of males, females may still spawn, although the unfertilized eggs will only remain attached to the pleopods for a short period (24 to 48 hours). In order to maximize their reproductive effort several decapods exhibit prolonged mate guarding, remove sperm from other males and use sperm-plugs (Bauer & Min, 1993; Elner & Beninger, 1995; Jivoff, 1997). However, most caridean shrimps do not show these behaviors and generally only pair for short periods to perform sperm transfer, with females being released shortly after (Bauer, 1976; Zhang *et al*., 2007). None the less, the studies by Thiel & Hinojosa (2003) on *Rhynchocinetes*

typus H. Milne Edwards, 1837, revealed that, at least in this rhynchocinetid species, females may discriminate sperm from different male morphs, namely by delaying spawning and removing sperm. In this way, females may influence the outcome of mating events and favor fertilization by sperm from dominant males. Using the same rhychocinetid species as a model, Van Son & Thiel (2006) also verified that, with multiple mating events, females become more selective (searching for higher quality mates), while males seem to conserve their reproductive resources in order to increase mate quantity.

As noted in the general description of the mating behavior of caridean shrimps, the endopods of the first two pair of pleopods of males are commonly modified from remaining pairs and it has been established that these structures function as gonopods. The endopod of the first pair usually differs in shape, size and setation, presenting cincinnuli (also known as coupling hooks) on its posterior end. The medial edge of the endopod of the second pair of pleopods bears a spinous process, the appendix masculinae, in addition to the appendix interna (which is also found in the remaining pleopods) (Figure 4.1) (Bauer, 1976, 1986). These structures seem to play an essential role in the success of spermatophore transfer during mating in caridean shrimps (e.g. Descouturelle, 1971; Berg & Sandifer, 1984). The disappearance of these secondary male sexual features in *Lysmata* shrimps, when shifting from male to simultaneous hermaphrodites (Bauer & Holt, 1998; Zhang & Lin, 2005a), do not seem to significantly affect their mating or fertilization ability (Zhang & Lin, 2004, 2005b).

The occurrence of protandric simultaneous hermaphroditism in the genus *Lysmata* appears to be highly advantageous, particularly for species living in pairs and displaying long-term pair-bonding (Rufino & Jones, 2001a) (e.g. *Lysmata amboinensis*, *L. debelius*, *L. grabhami*). The most evident benefit seems to be the ease with which these shrimps find mating partners compared with other pair-living shrimps, since 'any two will do' (Lin & Zhang, 2001a). Baeza (2006) notes that, at least for *Lysmata wurdemanni*, there appears to be an ontogenic change in the relationship between sex-specific investment and reproductive success. This aspect reveals an adaptive adjustment of sex allocation in order to improve the age-specific reproductive success of shrimps. However, the question arises as to why this remarkable mating system has not evolved outside the genus *Lysmata*, namely in those genera also exhibiting pairing behavior. Certainly alpheid shrimps occupying burrows as heterosexual pairs (Yanagisawa, 1984), pair-living harlequin shrimps of the genus *Hymenocera* (Seibt & Wickler, 1979), and non-gregarious symbiotic shrimps associating with specific hosts (e.g. *Periclimenes*) would also have a reproductive advantage if displaying this sexual system. Only future research studies seem likely to be able to clarify the origins of protandric simultaneous hermaphroditism and to establish why, if it is such an advantageous reproductive system, it is limited to such a restricted number of closely related species.

Presently, there is very little information available on embryo production and loss during the incubation period of marine ornamental shrimps. Calado & Narciso (2003a) verified that, as for many other caridean species, brood size increases with increasing body size in *Lysmata seticaudata*. Goy (1990) also verified a similar trend in the reproductive effort of other ornamental shrimps, namely *Lysmata 'wurdemanni'*, *Gnathophyllum americanum* and several *Periclimenes* species. Calado & Narciso (2003a) refer to the absence of senescence in the studied species. This feature may be detected in decapods if a negative allometry between brood size and body size is recorded (Bauer, 1991). Brood loss during the incubation period commonly occurs in caridean shrimps and it can be induced by aborted development, mechanical loss due to abrasion, maternal cannibalism, embryo predation and parasitism (Kuris, 1991). For *Lysmata seticaudata*, this loss may represent almost 15% of the total number of embryos. Calado & Narciso (2003a) point to mechanical stress induced by abrasion as the main cause for embryo loss in this species, since ovigerous shrimps continue to forage and to avoid predators by rapidly escaping through rocky cracks and rubble. Another probable cause of brood loss during the incubation period in marine ornamental shrimp species may be the continuous increase in embryo volume during the incubation period, a common feature in several decapod eggs (Morais *et al*., 2002; Rosa *et al*., 2007). This 'swelling' experienced by incubating embryos is mainly due to an increase in their membrane permeability before hatching, which results in a higher water uptake (Charmantier & Charmantier-Daures, 2001). In this way, since the physical space available in the abdomen for embryo incubation remains unchanged, it may become a limiting factor for embryo incubation during late development stages.

Chapter 5

Larval Development and Metamorphosis

5.1 Larval stages and morphology

In general, marine invertebrates are known to pass through complex life cycles comprising an embryonic, a larval, and a juvenile–adult phase (Pechenik, 1999). These organisms suffer radical ontogenic transitions of habitat and lifestyle, implying dramatic changes in their locomotion and feeding performance. Larvae display stage-specific adaptations to the pelagic environment, which are seen in their natatory and feeding appendages at a functional and morphological level (Williamson, 1982).

According to Anger (2006), a larva can be defined as a free-living early developmental stage, differing in specific traits from earlier and later life-history stages of the same species. These traits comprise morphological and behavioral features, which are mainly related to feeding and locomotion. In summary, they represent adaptations to a planktonic lifestyle, allowing larvae to exploit resources markedly different from those used by adults. While larval traits are transitory and stage specific, juveniles display underdeveloped adult traits (sometimes even lacking some), which will be only gradually completed during growth and maturation (Anger, 2006).

The most primitive crustacean larval form is the nauplius *sensu lato* (Scholtz, 2000; Waloszek & Maas, 2005), which has only three pairs of cephalic appendages (antennules, antennae and mandibles) with natatory functions. In the Dendrobranchiata, which include penaeid shrimps cultured worldwide for human consumption, the nauplius is followed by a different larval stage termed zoea. In the Pleocymata, which include marine ornamental stenopodidean and caridean shrimps, the larva does not hatch as a nauplius but as a zoea. A zoea is morphologically more advanced than a nauplius, swimming with thoracic appendages. The cephalic appendages of the zoea have lost their natatory functions (as exhibited by the nauplius), being mainly involved in food detection and in the perception of the surrounding environment. The zoeal phase is followed by a decapodid or megalopa stage (Williamson, 1982;

Anger, 2001). This stage is also referred to as a 'postlarva' (Felder *et al*., 1985), an ambiguous terminology commonly used by shrimp aquaculture professionals. Caridean and stenopodidean shrimp larvae exhibit a continuous pattern of change in their functional morphology, with successive stages displaying gradual rather than metamorphic changes. These shrimps also display a high intraspecific variability in the number and morphological traits of their larval stages. The end of the caridean and stenopodidean larval phase may be theoretically defined by the final loss of the natatory function of the exopods of pereiopods (Anger, 2006). However, this developmental change often occurs gradually rather than as a metamorphosis. The shifts in functional morphology are commonly followed by overlapping behavioral transitions, from pelagic zoeal swimming to a mixture of near-bottom swimming and crawling during the decapodid or megalopa stage, and eventually to walking on benthic surfaces in early juveniles. In this way, it is sometimes very difficult to clearly distinguish between late decapodids and early juveniles.

Species living in highly specialized habitats, such as freshwater decapods, as well as those living in close association with other organisms (obligate symbionts), may sometimes display abbreviated larval development (Powell, 1979; Saito & Konishi, 1999). This development type can be further divided in advanced development, with larvae hatching as a more developed zoea than that usually recorded in congeneric relatives, and direct development, when no free-swimming larval stages are produced and newly hatched individuals already display an adult-like appearance. This development pattern is commonly displayed by species which would not benefit from dispersing too far from their habitats or host populations (Duffy, 2002; Bolaños *et al*., 2004). Caridean and stenopodidean shrimps available in the aquarium trade display larvae sharing the following morphological features (after Dos Santos & González-Gordillo, 2004): a laterally flattened carapace and a flattened telson without a median spine. However, stenopodidean shrimps differ from caridean larvae by the second telson's spine assuming the form of a fine seta (commonly referred to as an anomuran hair) and by the presence of small dorsal spines in the first abdominal segment, a long median dorsal spine in the third abdominal segment and ventral hook-shaped spines in the fifth abdominal segment (Figures 5.1 and 5.2).

The stenopodidean and caridean shrimps commonly traded have a large number of larval stages, making larval staging a challenging task. The main difficulty for correctly staging these larvae is that morphology of a given 'instar' (or 'numerical stage', defined by the number of larval moults) varies among hatches, individuals, and rearing conditions (Anger, 2001). Larvae may display one or a combination of all three subcategories of delayed development in their larval history: mark-time moulting, and intercalated and terminally additive staging. All these events increase the pelagic larval phase of the species (as defined by Gore, 1985). Intercalated staging is a simple type of delayed larval development, which can be defined through the insertion of

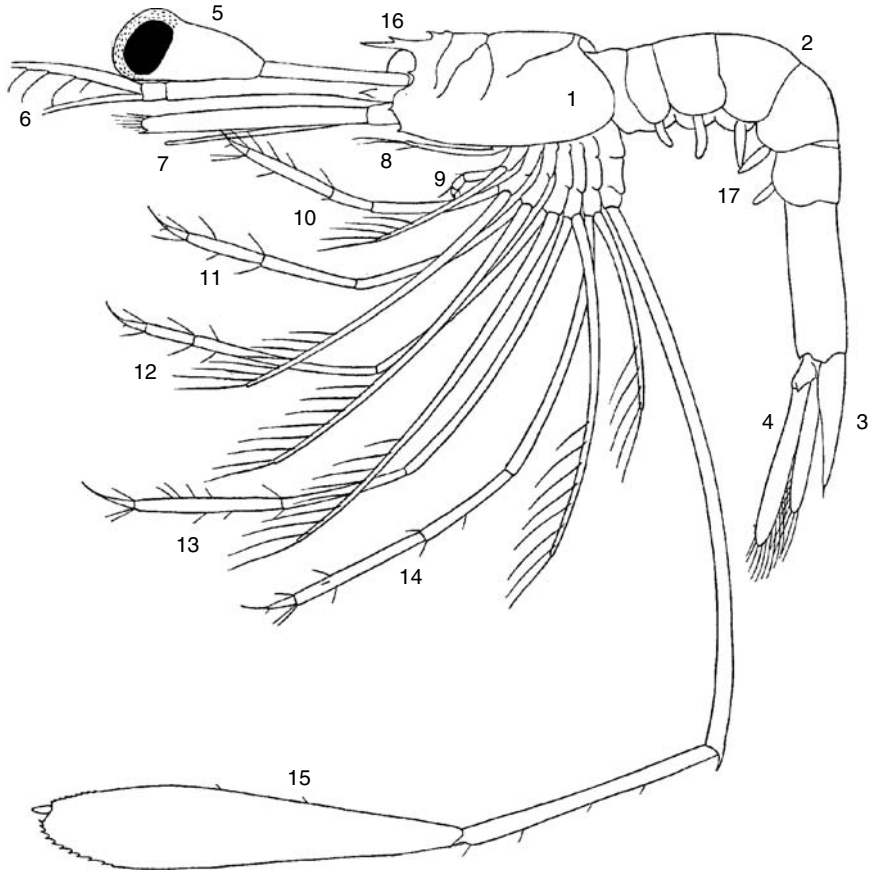


Figure 5.1 General larval morphology of a caridean zoeal stage using a 'typical' *Lysmata* larvae as an example: 1, carapace; 2, abdominal segments; 3, telson; 4, uropods (inner and outer branches); 5, stalked eye (fused with the carapace in newly hatched larvae); 6, antennules; 7, antenna; 8, first maxilliped; 9, second maxilliped (note exopod displaying long natatory setae); 10, third maxilliped (note exopod displaying long natatory setae); 11, first pereiopod (note exopod displaying long natatory setae); 12, second pereiopod (note exopod displaying long natatory setae); 13, third pereiopod (note exopod displaying long natatory setae); 14, fourth pereiopod (note exopod displaying long natatory setae); 15, fifth pereiopod (*Lysmata* larvae display this appendage elongated with a flattened paddle-like propodus); 16, rostrum (may already display rostral spines); 17, pleopods (these structures never display natatory functions in zoeal stages) (adapted from Gurney, 1937).

extra stages between those normally occurring in a species' developmental series. Intercalated stages appear as distinct instars, morphologically differing from the preceding one and from the 'normally' following stage. The most common variations recorded are related to larval size, appendage morphology, setation formulae, anatomical development or a combination of all these features. It is now accepted that the occurrence of intercalated stages is a larval response to unfavourable conditions, such as starvation, thermal or saline

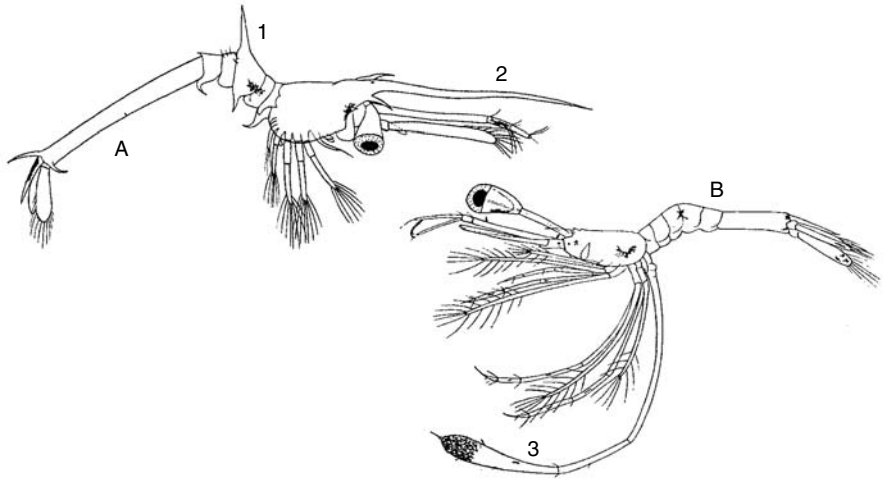


Figure 5.2 'Typical' *Stenopus* zoea (A) and *Lysmata* zoea (B); commonly damaged larval appendages during culture: abdominal spines, namely the large dorsal one (1), rostrum (2) and the elongated paddle-like fifth pereiopod (3) (larvae not to scale) (adapted from Gurney, 1937).

stress, and pollution (Gore, 1985). Mark-time moulting has long been recorded in laboratory cultures of caridean and stenopodidean shrimps (Calado *et al*., 2001a). It occurs when a zoeal stage enters a sequence of moults in which very little change in morphology takes place, even if it exhibits some small increases in larval size. Larvae may continue 'marking time' for relatively long periods, eventually dying or reassuming their normal moulting pattern and completing larval development. The genus *Rhynchocinetes* is well known to display this type of moulting pattern (Gurney & Lebour, 1941). In fact, there are records of 'giant' zoeal caridean and stenopodidean larvae occurring in the plankton that are several millimetres larger than the 'typical' last zoeal stage (Williamson, 1970, 1976). When analysing material collected from plankton samples, Gurney & Lebour (1941) recorded giant stenopodid larvae moulting to megalopa with a length of 21 mm. However, in the same samples, larvae from the same species but 31 mm long were still in a zoeal stage. Apparently, unsuitable food levels may be responsible for this morphogenic delay, whereas moulting and growth seem to continue their normal pattern (Knowlton, 1974). If so, mark-time moulting can be labelled as a maintenance mode, with energy being used simply to keep the larva alive, and once the energetic requirements are fulfilled, growth first and morphogenesis later may resume their normal mode. Finally, terminally additive staging shows several similarities with mark-time moulting, since supernumerary stages are also produced. It can be defined as a particular case of mark-time moulting, which only takes place at the end of a developmental sequence. It is characterized by the occurrence of relatively similar stages, which differ mainly in size and display minor morphological differences. Terminally additive staging may be

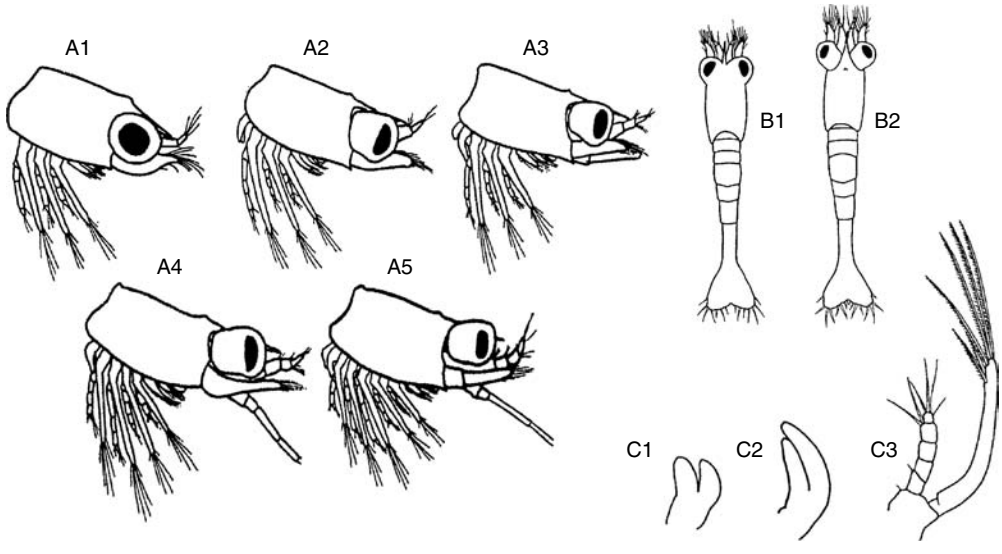


Figure 5.3 Relevant morphological features for staging caridean and stenopodidian zoeae: A1–5, development sequence of pereiopods; B1, eyes sessile (first zoeal stage); B2, eyes stalked (second and following zoeal stages); C1–3, development sequence of pereiopods; C1, small biramous pereiopod bud; C2, larger biramous pereiopod bud; C3, functional pereiopod (in some species the pereiopods may become functional every two stages instead of every three). Drawings are not to scale.

triggered by unsuitable food levels, stressful temperatures or salinities (Gore, 1985), or in certain species the lack of specific settlement cues (e.g. certain *Periclimenes* species late-stage zoeae need the chemical cues released by their host sea anemone to trigger metamorphosis) (Goy, 1990; Dos Santos *et al*., 2004).

Despite the difficulties in accurately staging caridean and stenopodidean shrimp larvae (Figures 5.3 and 5.4), there are growing efforts to improve the accuracy and standardization of larval descriptions (Clark *et al*., 1998), as well as to provide complete larval series descriptions and scientific literature revisions on this topic (e.g. González-Gordillo *et al*., 2001). These aspects coupled with several technical improvements in the raising of decapod larvae in captivity (e.g. Sastry, 1970; Ingle & Clark, 1977; Illingworth *et al*., 1997; Kittaka, 1997; Ritar, 2001; Calado *et al*., 2003a) have allowed us to better understand the larval biology of marine ornamental shrimps.

The following features are only indicative, and are provided as guidelines to help in the staging of caridean or stenopodidean larvae using the complete larval series of *Lysmata seticaudata* (displaying nine zoeal stages) as described by Calado *et al*. (2004):

- First zoeal stage: eyes fused with carapace; telson with 7 + 7 setae.
- Second zoeal stage: eyes stalked; telson with 8 + 8 setae.
- Third zoeal stage: uropods are biramous, with well developed exopods and small endopods.

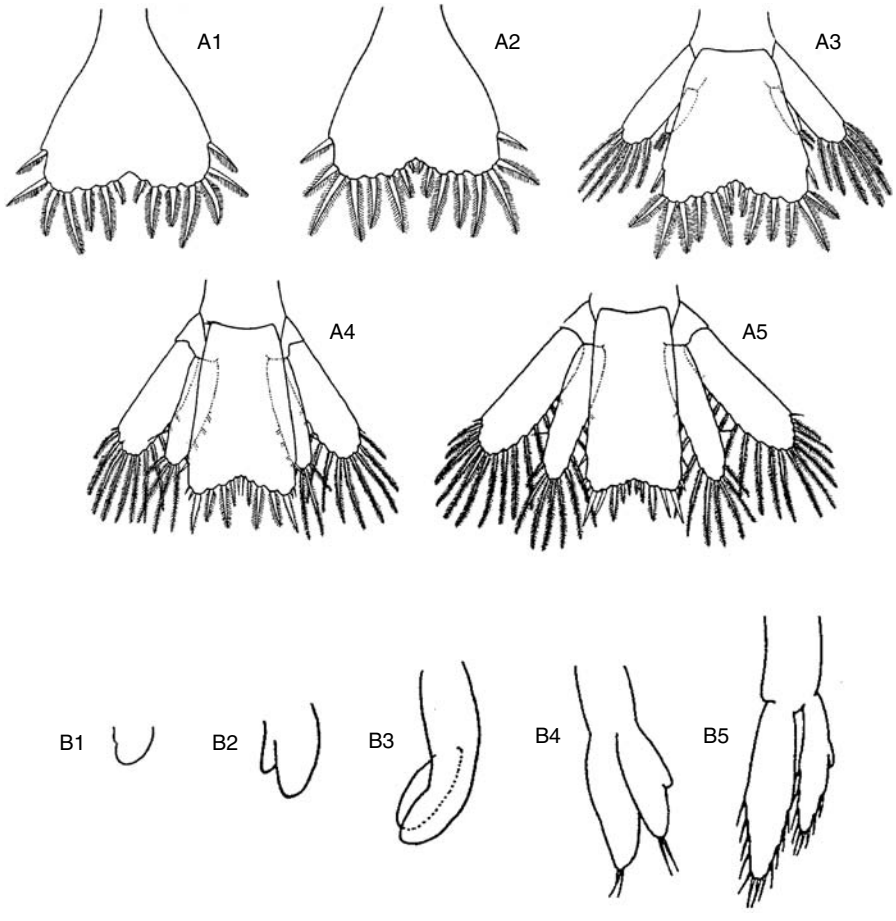


Figure 5.4 Relevant morphological features for staging caridean and stenopodidian zoeae: A1, telson presenting seven spines (first zoeal stage); A2, telson presenting eight spines (second zoeal stage); A3, inner branch of uropods rudimentary (third zoeal stage); A4, inner branch of uropods shorter than telson (fourth zoeal stage); A5, inner branch of uropods almost as long as (or even longer than) the telson (fifth zoeal stage); B1, uniramous pleopod buds; B2, biramous pleopod buds; B3, biramous pleopods (antepenultimate zoeal stage); B4, biramous pleopods sparsely setose (penultimate zoeal stage); B5, biramous pleopods densely setose (last zoeal stage). Drawings are not to scale.

- Fourth zoeal stage: the exopod of uropods is longer than the telson and the endopods are now fully developed.
- Fifth zoeal stage: the exopod and endopod of uropods are longer than the telson.
- Sixth zoeal stage: all pereiopods are functional; pleopods present as small buttons.
- Seventh zoeal stage: pleopods present as biramous buds.
- Eight zoeal stage: pleopods with the endopod bud-like with few setae and a rudimentary exopod.

- Ninth zoeal stage: pleopods with endopod and exopod fully developed and displaying numerous setae; small appendix interna present in the endopod from second to fifth pair of pleopods.

The morphological features selected above are the ones that can be more easily observed with the help of a dissecting microscope. This aspect is of particular importance when staging live shrimp larvae, namely later zoeal stages, which are extremely frail. Excessive manipulation of these later zoeal stages commonly damages the pereiopods or rostrum, which will later be potential open doors for infections by pathogenic agents. Even if a larva survives extensive manipulation, the morphological damage induced may be enough to disrupt the 'normal' larval cycle and lead to the appearance of extra instars. The ontogeny of larval pereiopod development is not so easily recorded without extensive manipulation, but is a very important feature for the diagnosis of early zoeal stages. A pereiopod present in a larval stage as a uniramous bud will usually be present in the following stage with a biramous appearance. A biramous pereiopod bud will commonly appear in the following zoeal stage as a fully functional pereiopod. On the other hand, the ontogeny of larval pleopod development is very important in the staging of late zoeal stages. Usually, only in the zoeal stage in which all pereiopods become functional will the pleopods be visible for the first time, normally as small buttons. The antepenultimate zoeal stage generally displays pleopods in the form of biramous buds whereas the penultimate stage shows bud-like endopods with a few simple setae. The last zoeal stage displays fully developed pleopods with numerous setae on the endopod and exopod.

The number of zoeal stages and their special morphological features in the most traded marine ornamental shrimp genus are summarized in Table 5.1.

Intra- and interspecific competition among larvae has been identified as a potentially important biotic selection force (Strathmann, 1996). Apparently, competition for food in the plankton is more significant at higher trophic levels (Verity & Smetacek, 1996) and decapod larvae display several adaptations to minimize the effects of predation. Preferential hatching of decapod larvae during the night period seems to indicate the important selective force exerted by predation pressure (Anger, 2001). Another interesting example of potential predation pressure, induced by visual predators, is the synchronization of larval moulting around dawn or the night period. Cues from natural light cycles seem to be responsible for this synchronization in moult timing (Mikami & Greenwood, 1997; Matsuda *et al.*, 2003). Long larval spines have for long been considered as morphological adaptations enhancing buoyancy and considerably minimizing the energetic costs associated with larval swimming (Grünbaum & Strathmann, 2003). However, growing evidence now seems to point to an additional role of large spines in reducing predation (Morgan, 1989, 1990). Morgan (1992) also highlighted how large carapace spines and body size are more effective in avoiding predation by planktivorous fishes

Table 5.1 Number of zoeal stages and diagnosing morphological features of the most commonly traded marine ornamental shrimp species

Family	Genus	Number of stages	Diagnosing morphological features	Relevant references
Stenopodidae	*Stenopus*	9 zoeal stages?[1]	Long rostrum, first abdominal somite with dorsal spines, third with a long dorsal spine and fifth with a ventral hooked spine	4
Alpheidae	*Alpheus*	Up to 9 zoeal stages; sometimes abbreviated development may occur	Slender and elongated body; short needle-like rostrum; fifth pereipods precociously developed, being very long and slender	5
Gnathophyllidae	*Gnathophyllum*	Unknown	S-shaped body	6
Hippolytidae	*Lysmata*	At least 9 zoeal stages, usually more	Long eyestalks, penultimate segment (propodus) of the fifth pereiopod (sometimes also the third and fourth) largely flattened and paddle shaped	7
	Lysmatella	Unknown	Unknown	—
	Parhippolyte	Unknown	Unknown	—
	Saron	4 zoeal stages?[2]	Stout built body; newly hatched larvae with the rostrum slightly pointing downwards	8
	Thor	6 zoeal stages	Rostrum absent in the first stage and later present as a blunt triangle	9

Hymenoceridae	*Hymenocera*	12 zoeal stages?[3]	S-shaped body	[10]
Palaemonidae	*Periclimenes*	8 zoeal stages	S-shaped body; long and serrated supraorbital spine	[11]
	Urocaridella	Unknown	S-shaped body	—
Rhynchocinetidae	*Rhynchocinetes*	10–11 zoeal stages	Newly hatched larvae with a well developed rostrum slightly pointing down; dorsal portion of the third abdominal segment may present a projection	[12]

[1] Gurney & Lebour (1941) suggest this number of zoeal stages for stenopodideans but point out that further studies are required to confirm this hypothesis.
[2] Some zoeal stages may have been overlooked by Sankolli & Kewalramani (1962).
[3] Extra zoeal stages due to mark-time moulting may have been recorded by Fiedler (1994).
[4] Gurney, 1936; Williamson, 1976; Seridji, 1990.
[5] Gurney, 1942; Barnich, 1996.
[6] Bruce, 1986.
[7] Gurney, 1937, 1942; Wunsch, 1996; Calado *et al.*, 2004.
[8] Gurney, 1937; Sankolli & Kewalramani, 1962.
[9] Lebour, 1940; Broad, 1957; Dobkin, 1968; Yang & Okuno, 2004.
[10] Fiedler, 1994.
[11] Gurney, 1938a; Gore *et al.*, 1981; Dos Santos *et al.*, 2004.
[12] Gurney & Lebour, 1941; Maihara & Kyoya, 2001.

than by invertebrate predators. This is related to the fact that planktivorous fishes swallow their prey whole and the mouth aperture is a limiting factor to prey ingestion. In general, it seems that physical resistance to predators is more common than chemical resistance (Bullard *et al*., 1999). The lack of pigmentation in larval stages has also been pointed to as a mechanism that makes visual detection by predators difficult (Jeffs *et al*., 2001). However, decapod larvae commonly display more or less conspicuous chromatophores, and the laboratory experiments by Morgan & Christy (1997) did not reveal a significant influence of larval pigmentation on the vulnerability to predation by planktivorous fish. The work by Rufino & Jones (2001b) even suggests that the conspicuously pigmented fifth pair of pereiopods of *Lysmata* larvae may be their first line of defense against predation. These paddle-shaped pereiopods display large red ocelli in their flattened propodus, which can easily be lost under mechanical stress and regenerated in the next moult. Being highly conspicuous, these structures can be a preferential target for visual predators over other vital body parts, namely the carapace area. In an apparently similar strategy, several shrimp larvae also display conspicuous ocelli in the outer branch of their uropods, somehow mimicking the anterior area of their carapace, where larval eyes are located (Calado, unpubl. obs.). The 'shadow response' is also an antipredatory mechanism exhibited by decapod larvae, resulting in a fast escape response when light intensity decreases abruptly (Forward, 1977, 1986). In larval shrimps this escape response is commonly expressed by single, or a series of, fast contractions of abdominal muscles (decreasing the angle formed by the curved abdomen), which projects the larva a few centimetres backwards (Rufino & Jones, 2001b). Forward & Rittschof (2000) and Cohen & Forward (2003) have shown that newly hatched zoeae of the mud crab *Rhithropanopeus harrisii* (Gould, 1841) are able to detect kairomones (chemical signs released from predatory fishes or invertebrates). However, further research is required to ascertain whether larval shrimps also display this ability and if it experiences any ontogenic variations during larval development.

5.2 Energetic aspects of larval biology

The need for planktonic food displayed by most decapod larvae is termed planktotrophy. However, some decapod species may display during their entire larval development, or at least during part of it, a different mode of development, when they commonly rely on endogenous reserves. This development mode is termed lecithotrophy and it is considered as an adaptation to highly variable or low food production habitats (Thorson, 1950; Pond *et al*., 1997; Gimenez & Anger, 2005). This larval adaptation is certainly of major relevance, since food limitation to planktotrophic larvae can seriously affect species recruitment (Strathmann, 1987; Olson & Olson, 1989). Although planktotrophy

and lecithotrophy are sometimes considered as alternative strategies (Poulin *et al*., 2001), in decapod larvae these seem to be the opposite extremes of a continuum of developmental patterns (Anger, 1995). Larvae displaying primary lecithotrophy rely on the parental energy invested in reproduction, commonly in the form of remaining egg yolk reserves, to fuel their development (Anger, 2001). In marine ornamental shrimps the occurrence of highly enhanced yolk reserves that enable the larvae to undergo a completely non-feeding development (full lecithotrophy) has never been recorded. However, some ornamental shrimp species may display moderately enhanced yolk reserves, allowing newly hatched larvae to advance to the next zoeal stage even in the complete absence of food. It is important to highlight that these larvae retain their ability to capture and ingest available dietary prey. This highly flexible mode of development is termed facultative primary lecithotrophy (Thessalou-Legaki *et al*., 1999; Calado *et al*., 2005a, b). Secondary lecithotrophy is generally recorded in late non-feeding larval stages, which moult to a juvenile (that resumes the feeding activity). However, the endogenous reserves being used to energetically support this development result from plankton-derived organic matter accumulated by earlier larval stages (Anger, 1989; McWilliam & Phillips, 1997; Booth *et al*., 2005; George, 2005). Although secondary lecithotrophy was previously only recorded in non-feeding late larval stages, Calado *et al*. (2007b) reported that the megalopa of *Lysmata seticaudata* is able to moult to the juvenile stage in the absence of food, while retaining its feeding ability. This mode of development has been termed facultative secondary lecithotrophy. Calado *et al*. (2007c) have also verified the existence of facultative secondary lecithotrophy in the second zoeal stage of several *Lysmata* species produced from fed newly hatched larvae, which were able to moult to the third zoeal stage in the total absence of food. Apparently, the second zoeal stage fuels its development through the catabolization of energetic reserves accumulated through dietary prey ingestion by the first zoeal stage. This hypothesis is supported by the absence of facultative secondary lecithotrophy in zoea II produced from starved zoea I.

Planktotrophic decapod larvae must start feeding immediately after hatching, in order to avoid deleterious effects induced by prolonged starvation periods. Anger & Dawirs (1981) have experimentally demonstrated the occurrence of a critical period in the larval development of decapods termed the 'point of no return'. The 'point of no return' represents a threshold at which larvae which were exposed to starvation, and were subsequently fed, may remain alive for a variable period of time. They are, however, incapable of recovering from the early nutritional stress imposed, and do not develop any further and finally die. Additionally, these authors recorded the existence of another critical period termed as 'point of reserve saturation'. The 'point of reserve saturation' is a threshold beyond which food uptake is no longer essential for the larvae to be able to develop and moult to the following stage. Understanding these critical points in early larval feeding can be valuable in establishing

successful aquaculture protocols for commercially valuable decapods (Paschke *et al*., 2004). Preliminary studies identifying the 'point of no return' and the 'point of reserve saturation' in early zoeal stages may help to minimize mortality and increase the survival to metamorphosis of marine ornamental shrimp larvae. The importance of these studies is even more relevant if we consider that the 'point of no return' and the 'point of reserve saturation' can be experimentally quantified, clarifying the nutritional flexibility of planktotrophic decapod larvae (Sulkin & van Heukelem, 1980; Sulkin *et al*., 1998; Gimenez & Anger, 2005).

5.3 Metamorphosis and settlement cues

The larval period of a decapod larva may be divided into two different phases: (1) a pre-competence phase, where the larva is still not able to metamorphose, commonly functioning for dispersal and growth; (2) a final phase of competence, in which the larva is physiologically and morphologically receptive to cues that may trigger metamorphosis (Pawlik, 1992; Pechenik, 1999). Metamorphosis is an inherently integrative concept, highly relevant to developmental biology and ecology. Although several definitions of metamorphosis are usually employed, it can be described, in the strict sense, as a particular life history transition from a larval to a juvenile (or adult) stage, accompanied by dramatic changes in morphology, physiology and ecology (Bishop *et al*., 2006). In several decapod larvae this process can be triggered by chemical waterborne cues from adult conspecifics and/or the habitat, as well as by physical cues (Crisp, 1974; Burke, 1983; Gebauer *et al*., 2002, 2003). Sometimes, larvae may not respond to a single particular stimulus but rather to a combination of various stimuli, which ultimately promote a stronger effect in the triggering of metamorphosis than an isolated stimulus (O'Connor 1991; Gebauer *et al*., 1998). In the absence of specific cues, competent larvae may delay metamorphosis, from just a few hours to several days (Pechenik, 1990). In general, this delay is concluded by the occurrence of a 'spontaneous' metamorphosis, even in the absence of specific cues, or else results in the death of the larvae (Gebauer *et al*., 1998).

Physical and chemical cues are detected by larval mechano- and chemoreceptors, respectively. Mechanoreceptive setae (also known as *sensillae*) are frequent integumental organs present in decapod larvae, displaying a moveable socket at the base and various other cuticular components that are produced by enveloping cells (Felgenhauer, 1992). Some decapod larvae (e.g. the phyllosoma of spiny lobsters) may display up to seven different types of integumental mechanoreceptors (Nishida & Kittaka, 1992). Chemoreceptors responsible for detecting chemical cues such as pheromones and kairomones are commonly located on the body surface of decapod larvae (Govind, 1992). There are two

major types of chemoreceptor setae, namely with and without apical pores. Pore-type chemoreceptors are typically found in the aesthetascs, which are modified setae with sensory functions, present in the antennules of larval decapods (Laverack, 1988).

The flexibility exhibited by decapod larvae in the timing of metamorphosis has been considered as a remarkable selective advantage that enhances the probability of a late larval stage locating a suitable habitat for juvenile and adult survival (Thorson, 1950; Obrebski, 1979; Gebauer *et al*., 2003). However, delaying metamorphosis also has costs for developing larvae, namely if it results in the production of poor quality and/or smaller juveniles (Harvey, 1992; Gebauer *et al*., 1999). This particular aspect of larval biology may prove to be highly relevant for the culture of marine ornamental caridean and stenopodidean shrimps since these larvae are known to be able to delay metamorphosis for several weeks. If, after long larval culture periods, only poor quality juveniles are produced that display low survival and growth rates, culture profitability will be seriously threatened.

Chapter 6

Broodstock Maintenance in Captivity

6.1 Broodstock maturation systems

The commercial-scale culture of marine ornamental shrimps can only be possible if a large number of high quality larvae are regularly available. One way to achieve this objective is to ensure a permanent supply of wild ovigerous shrimps, which will later release their larvae in captivity. However, relying only on the collection of wild shrimps will always be a risky option because of the unpredictability of wild specimen collection and the fact that it is considered to be a potentially unsustainable solution. Therefore, a feasible way to ensure regular production of ornamental shrimp larvae, either for research studies or for commercial ventures, is to keep breeding pairs in captivity and induce their reproduction.

Commonly, ornamental shrimps are kept as mated pairs in isolated aquariums, requiring optimal husbandry conditions to trigger gonad maturation. Assuring 'good water quality' in broodstock maturation systems is the first requirement to keep reproducing shrimps successfully in captivity. The general concept of 'good water quality' is achieved by employing efficient mechanical, chemical and biological processes that ensure a stable and oligotrophic environment. This can be achieved in open systems installed in facilities operating on coastlines, into which natural sea water is pumped, circulated through broodstock tanks and returned to the coast again. However, the negative environmental image of onshore aquaculture facilities, current legislation restrictions on the implementation of these units, high land cost and the growing conflicts of interest in the use of the coastline commonly force aquaculture enterprises to set their facilities away from the sea and employ inland recirculated systems.

In certain situations, the distance to a reliable source of sea water may economically impair the use of natural marine water. In these situations, artificial sea water is commonly employed, relying on a mix of high-grade synthetic sea salts with purified fresh water (e.g. by reverse osmosis or

demineralization units). Recirculated systems always display high efficiency filtration equipment in order to extend the use of recirculated water. The successful design of these systems must completely fulfil the needs of the organisms being kept in captivity, while minimizing the costs associated with their construction and operation. The reduced size of most marine ornamental shrimps, when compared with other decapod crustaceans cultured for human consumption, allows the design of compact systems which maximize production and occupy a reduced space. Obviously, this approach considerably decreases the system's construction costs. The major components used in recirculating systems include:

(1) broodstock aquariums of variable sizes (because of the variable dimensions of ornamental shrimps and the existence of more or less pronounced agonistic behaviors towards conspecifics);
(2) water pumps (to ensure good water circulation);
(3) mesh bags (varying from 20 to 400 μm in mesh size and employed for solids removal);
(4) activated carbon and various other types of resins (e.g. antiphosphate) (employed to remove dissolved pollutants);
(5) trickling filters, fluidized filters, live rock or coralline sand (to ensure biological filtration);
(6) heating and cooling units (to minimize temperature fluctuations);
(7) protein skimmers (to remove dissolved organic compounds so as to prevent deterioration of water quality);
(8) 'kalkwasser' reactors (to dose a saturated solution of calcium hydroxide which regulates pH variations and ensures optimum calcium concentrations);
(9) osmoregulators (to minimize salinity changes by dosing purified fresh water to compensate for evaporation);
(10) nitrate reactors (to recreate an anaerobic atmosphere that favours the development of anaerobic bacteria capable of reducing nitrate to atmospheric nitrogen);
(11) ultraviolet sterilizers (to control potential pathogens); and
(12) ozonizers (to control potential pathogens and oxidize organic compounds).

Broodstock tanks should preferably be made of transparent materials (glass, plastic or acrylic) in order to allow the proper monitoring of reproductive shrimp pairs. The regular observation of broodstock shrimps is important to detect the existence of ovigerous females, to monitor embryonic development, to detect newly hatched larvae and to inspect the general health condition of the broodstock. When pairing species with pronounced agonistic behavior towards conspecifics (e.g. pair-bonding *Lysmata* (Rufino & Jones, 2001a) and *Stenopus* (Johnson, 1969, 1977)), transparent broodstock tanks allow the

detection of warning signs by the aggressor shrimp before serious damage is inflicted on a conspecific.

Mechanical filters require regular cleaning to maximize their performance since negligent maintenance may allow the excessive accumulation of detritus and even contribute to an accelerated decrease in water quality.

Chemical filters are replaced after being used for a certain period, usually to solve a specific problem (e.g. an unusually high concentration of phosphates). When saturated, the media employed for chemical filtration must be replaced since there is a risk that trapped compounds may leach back into the water. The status of chemical filters is more perceptible when employing media that change color after being saturated. Nevertheless, the wisest approach will always be to perform water tests and monitor the existence of the compounds that were supposed to be removed by chemical filtration. In addition, it must also be stressed that certain media (activated carbon) are less specific than desired and may be able to trap desirable compounds, namely trace elements (e.g. strontium and iodine).

The biological filtration capacity of recirculated systems should be designed and dimensioned to effectively keep nitrogenous compounds as close to zero as possible. The presence of ammonia and nitrite in recirculated water is highly deleterious for marine invertebrates, as are high concentrations of nitrate for marine shrimps (Muir *et al.*, 1991). Trickling and fluidized filters are known to be highly efficient in the nitrification of waste products (Sastry *et al.*, 1999; Eding *et al.*, 2006). However, the denitrifying action of these biological filters, if present at all, is always considerably reduced and may lead to a build-up of nitrate levels. In these situations nitrate may be exported by performing regular water changes (diluting the concentration of this nitrogenous compound) or by using macroalgae (which will use nitrate as a source of nitrogen for their metabolism). Sediment-based biological filtration is also used in some recirculating systems, and is widespread practice among marine aquarium keepers. Commonly, a coarse or thin 'sandbed' of carbonate sediments, of variable depth, is placed on the bare bottom of the aquarium to perform biological filtration. The shallower areas of sandbeds are in contact with oxygenated water and become ideal areas for the development of large bacterial colonies responsible for nitrification. In the deeper areas, sandbeds generate an anoxic environment, since the oxygen present in the water reaching this area has already been used in the nitrifying processes occurring in the upper layers. This anoxic atmosphere allows the development of denitrifying anaerobic bacteria. Toonen & Wee (2005) did not find any significant differences in the performance of deep (9.0 cm) or shallow (2.5 cm) beds using either coarse (2 mm) or fine (0.2 mm) sediment particles. Nevertheless, when employing a sandbed in broodstock tanks it may be preferable to use shallow beds of coarse sediment in order to allow proper siphoning of certain waste products (e.g. uneaten food particles). Highly porous coralline rock, commonly known as live rock, is used in marine aquariums for its aesthetic value

and for the rich variety of invertebrates and algae that it harbours. Additionally, the rich community of microorganisms and their high area/volume ratio make them a valuable option for biological filtration. Nitrification occurs in the outer regions of live rock, which are surrounded by water with high levels of oxygen. On the other hand, denitrification is believed to occur in the inner parts of live rock. The occurrence of denitrification is assumed since surrounding water will slowly penetrate the highly porous carbonate material of live rock and will eventually reach its inner core already depleted of oxygen, which will create an anoxic environment that favours the development of denitrifying bacteria. Although live rock can be very effective in ensuring biological filtration, its high market value can make its use prohibitive in commercial-size recirculated systems.

Water temperature control plays a vital role in aquaculture management (León *et al*., 2006). Heating and cooling units are essential to stabilize temperature and ensure optimal conditions to trigger the maturation of gonads and the incubation of embryos. The direct effect of temperature on the moulting frequency of shrimps kept in captivity is well documented (Benayoun & Fowler, 1980) and higher water temperatures are known to shorten the duration of embryogenesis (Tong *et al*., 2000; Wehrtmann & López, 2003). However, the viability of newly hatched larvae may be negatively affected if thermal fluctuations occur during the incubation period (Laughlin & French, 1989; Smith *et al*., 2002a).

Protein skimmers are considered essential equipment in modern marine recirculated systems, being indispensable for the effective removal of numerous organic compounds present in the water before these can start to break down and seriously affect water quality. These pieces of equipment are basically air reactors that maximize the mix of microbubbles of atmospheric air with sea water, and allow the hydrophobic portion of organic compounds to bond to these microbubbles. Physical processes linked to the collapse of the air bubbles produced allow the collection of trapped organic compounds in a cup placed in the top of the protein skimmer. The diameter and height of protein skimmers significantly affect their performance and, in order to be effective, they need to be suitably dimensioned according to the water volume to be treated and to the systems bio-load (Escobal, 2000). The possibility of 'over skimming' and removing important trace elements from recirculated sea water has been questioned several times. However, there is still no scientific evidence supporting or refuting this possibility.

Kalkwasser reactors are commonly employed in association with osmoregulators, and dose a saturated solution of calcium hydroxide ($Ca(OH)_2$) in purified freshwater (commonly by reverse osmosis units). This solution compensates for the water losses promoted by evaporation in recirculated systems, minimizing salinity variations. Apart from inducing osmotic stress in broodstock, salinity changes during the incubation period may also significantly affect embryonic and larval quality (Gimenez & Anger, 2001). The fast

dissociation of calcium hydroxide makes calcium (Ca^{2+}) and hydroxide (OH^-) ions available. While the first are important to meet the needs of decapod crustaceans for this mineral, namely during the moult (Wheatly, 1996, 1999; Wheatly *et al.*, 2002), OH^- ions are important to keep pH stable by reinforcing seawater buffering capacity.

On some occasions the denitrifying capacity of recirculated systems with high bio-loads may need to be reinforced by the use of nitrate reactors. Nitrate reactors are airtight containers filled with suitable media to promote the growth of denitrifying bacteria in an anaerobic environment. The use of immobilized *Pseudomonas* in a freeze-dried, alginate–starch matrix, with starch serving as a bacterial carbon source and a cellular matrix-strengthening filler, has proved to be very effective in removing considerable amounts of nitrate from sea water (Tal *et al.*, 2003). Nitrate reactors are commonly fed water from the recirculated system at a very slow flow rate, in order to prevent the input of oxygen. The presence of oxygen inside the reactor would disrupt the microbiological community harboured in the reactor's media. When operating such equipment it is important to monitor nitrate and nitrite concentrations of inflow and outflow water. Elevated nitrite levels can be produced if the water inflow to the reactor is too high, allowing only incomplete bacterial nitrate reduction to be performed.

Ultraviolet irradiation is widely applied in recirculated systems, with the primary objective of microbiological disinfection (Liltved *et al.*, 1995; Summerfelt, 2003). The most widespread type of UV bulb in the industry is low pressure, supplying an irradiation of 254 nm wavelength. UV light intensity is usually described in milliwatts per square centimetre and UV dose is expressed in milliwatt-seconds per square centimetre. The UV dose required to inactivate microorganisms can be highly variable depending upon the target organism and the required sterilization rate (Wedemeyer, 1996). In order to achieve the desired level of UV disinfection, it is necessary to ensure a minimum UV dose. This dose is the product of the UV light intensity, exposure time to this constant intensity and a transmittance factor. Factors that can significantly affect the actual UV dose are water flow rate, water volume within the UV vessel, lamp intensity and the UV transmittance of water (e.g. turbid waters will exhibit lower transmittance levels). The UV unit should be sized to account for an average 40% decline in bulb intensity that occurs over a 12-month expected lamp life. Regular cleaning of the protective quartz sleeve (enclosing the UV lamp) will also maximize the levels of UV irradiation effectively sterilizing the water.

Ozone is a strong oxidizing agent that is now widely used for water disinfection and quality improvements in recirculated systems, as well as to disinfect outflowing waters of aquaculture facilities and to prevent the potential release of pathogens (Rueter & Johnson, 1995; Summerfelt, 2003). Effective water ozonation requires ozone generation, transfer into solution, contact time for reaction and sometimes ozone destruction, ensuring that no toxic

residue reaches the tanks holding broodstock (Summerfelt & Hochheimer, 1997). Ozone is commonly generated by inducing electrical discharges to a gas containing oxygen. Enriched oxygen is often preferred to atmospheric air since ozone production is two to three times more energy efficient (Masschelein, 1998). Supplying protein skimmers with ozone instead of atmospheric air is not an unusual practice, mainly due to the high contact time this equipment promotes between microbubbles and the system water. Ozone oxidation can kill microorganisms, but this process requires that a certain dissolved ozone concentration is kept in contact for a given period with the water being treated. Therefore, the equipment employed as a contact vessel must provide the time necessary for ozone to react and destroy target microorganisms. Krumins *et al.* (2001) did not record any significant differences when comparing the efficiency of different ozone dosing regimes (continuous dosing over the entire day, or for only 12 or 6 hours) for the same amount of ozone being dosed in a recirculated system.

While the use of UV light does not produce any toxic residues, ozone reactions may form harmful by-products that can be highly toxic to marine organisms (Tango & Gagnon, 2003). In seawater systems, ozone reacts with bromide ions to form the toxic oxidants hypobromous acid (HOBr) and hypobromite ion (OBr^-) (Huguenin & Colt, 1989). In some situations, prolonged ozonation may further oxidize the hypobromite ion and promote the formation of bromate (BrO_3^-), another persistent and toxic compound. Monitoring the oxidation/reduction potential (ORP) has become a common indirect measure for the detection of excessive ozone levels. This method employs an ORP probe, which is attached to a meter and placed in the water being ozonized and gives the ORP in millivolts (Tango & Gagnon, 2003). However, Buchan *et al.* (2005) have pointed out that an accurate method to measure dissolved ozone in sea water is still to be developed, since current methodologies are unable to distinguish ozone from other oxidants found in recirculated water. The same authors recommend that a DPD (N,N-diethyl-p-phenylenediamine) 'total chlorine test' should be used to measure dissolved ozone levels, with measures being expressed as TRO (total residual oxidants) in terms of milligrams per litre of Cl_2. Excessive ozone may be eliminated through:

(1) water retention within tanks immediately after ozonation;
(2) the application of small doses of reducing agents (e.g. sodium thiosulphate);
(3) passing ozonized water through a forced ventilation packed aeration column;
(4) passing the water through activated carbon;
(5) dosing low levels of hydrogen peroxide; or
(6) contact with high intensity UV lights (catalysing the conversion of O_3 to O_2) (Summerfelt, 2003).

Keeping symbiotic shrimps and their invertebrate hosts in captivity may require the use of suitable illumination in broodstock tanks if the host species exhibits symbiotic zooxanthellae (typically unicellular yellow-brown dinoflagellate algae) (Kinzie *et al.*, 1984; Schlacher *et al.*, 2007). Metal halide lamps are probably the standard choice for providing adequate lighting to all organisms harbouring zooxanthellae that are commonly traded for reef aquariums, from hard and soft corals, to giant clams and sea anemones. Nowadays, it is also possible to choose other equally suitable and more economic lamps to fulfil the lighting needs of organisms with symbiotic zooxanthellae, namely the new T5 compact fluorescent lamps (Delbeeck & Sprung, 2005).

In early studies, keeping breeding pairs in individual aquariums, commonly set up as regular reef displays, was a popular option, with only a reduced number of researchers employing recirculated systems. When keeping breeding pairs in isolated aquariums, the development stage of incubating embryos must be surveyed daily in order to detect females carrying late-stage embryos about to hatch. Previously, once detected, these shrimps were captured and placed in mesh baskets inside larval rearing tanks until larval hatching (Palmtag & Holt, 2001). After releasing their larvae, the shrimps were returned to their aquarium, re-establishing the breeding pair. Apart from being time consuming, this approach also displays several other disadvantages:

(1) Water quality is commonly lower in individual aquariums than in recirculated systems fully equipped with suitable filtration.
(2) Monitoring embryo incubation in a fully decorated aquarium is far from being an easy task and it is not uncommon to overlook embryos about to hatch. Larvae hatching in an aquarium not equipped with a larval collector are easily cannibalized by broodstock.
(3) Collecting ovigerous females may induce stress, sometimes resulting in egg loss.
(4) In species displaying pair bonding (e.g. *Stenopus* and some *Lysmata* species), the link between both members of the pair can be broken after a prolonged separation, triggering strong agonistic responses between the members of the breeding pair once they are reunited (Johnson, 1969, 1977; Rufino & Jones, 2001a).
(5) Inducing osmotic and thermal stress to females and late-stage embryos when placing them inside the larval rearing tank may disrupt their moulting and hatching mechanisms, respectively.
(6) Predation by females on their newly hatched larvae, if kept in the same container without a physical barrier to separate them.
(7) Females can moult soon after larval release, while still separated from their breeding partner. Depending on the species' post-moulting behavior (see Zhang *et al.*, 2007), the timing to successfully mate and produce a new embryo batch may be lost.

(8) Routine tasks, such as water quality monitoring, broodstock inspection and water changes take considerably more time. In addition, breeding pairs from the same species stocked in different isolated aquariums may display significant differences in embryo production and larval quality, mainly due to water quality variation between aquariums. This may be an important obstacle for experimental studies addressing ornamental shrimp larval culture, since the production of uniform larval pools may not be assured.

The recirculated maturation system for ornamental decapods described by Calado *et al*. (2007c) has solved most of the problems described above when keeping broodstock in captivity, and has become a valid choice for commercial and research purposes addressing marine ornamental shrimps (see color plate 6) (Figure 6.1). The main breakthroughs of this system are:

(1) the shorter periods of time required to perform routine tasks;
(2) the promotion of better water quality for broodstock keeping, because of the higher water volumes and better filtration systems;
(3) the fact that ovigerous females do not need to be captured before larval release and, therefore, the risk of disrupting reproductive pairs is minimized, as is the possibility of females moulting before being paired again;
(4) it separates newly hatched larvae from the reproductive pair, preventing adults from cannibalizing their larvae; and
(5) it allows live prey to be provided to larvae immediately after hatching, eliminating their exposure to starvation periods.

Newly hatched larvae can be quickly separated from the breeding pair if a movable plastic mesh is used to separate the breeding tank into two distinct areas. By using a unidirectional flow (from the breeding pair to the larval collection area), as well as by placing a source of light in the larval area, newly hatched larvae will actively swim away from parental shrimps in favour of the water current and towards the light. The positive phototaxis of decapod larvae has been well documented by several authors (e.g. Sulkin, 1975; Bigford, 1979; Adams & Paul, 1999). This larval feature may allow researchers to further improve the performance of larval collectors designed for maturation systems, minimizing the period of time during which newly hatched larvae remain in contact with parental shrimps. Larval hatching commonly occurs during the night, and light sources need to be switched on to attract the larvae to the collectors. However, breeding pairs commonly display a strong negative phototaxis and are much more active in darkness. For this reason actinic lights should be preferred over white ones in order to attract newly hatched larvae, since broodstock is more tolerant of this type of illumination. Mesh screens should be attached to the outflow in the larval collection area to

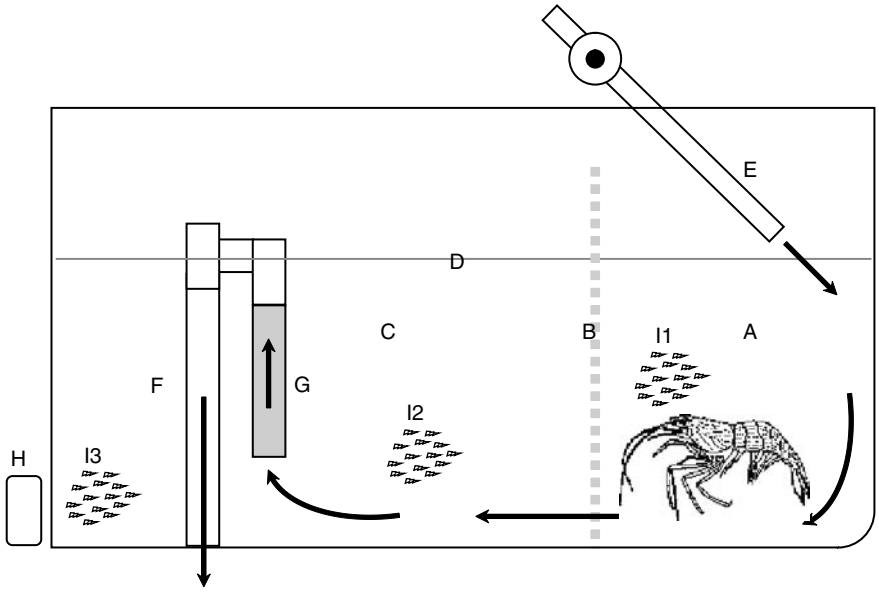


Figure 6.1 Maturation tank: A, broodstock shrimp area; B, large, rigid and removable mesh division to prevent broodstock from entering the larval area; C, larval area; D, water level; E, inflow; F, standpipe acting as water outflow and regulating water level inside the maturation tank (notice the PVC 'T' at the top that will act as an overflow should the mesh screen somehow become clogged); G, mesh screen (500 μm mesh to prevent newly hatched larvae from being drained; or 150 μm mesh to prevent newly hatched *Artemia* nauplii from being drained if this larval prey is added to the tank to prevent shrimp larvae from starving since hatching until they are placed in the larval rearing system); H, light source (preferably actinic light); I, shrimp larvae; I1,hatching, I2, being transported to the larval area, and I3, concentrating near the light source; black arrows represent predominant water flow.

prevent larvae from being lost. Mesh size in these screens is usually 500 μm and the mesh commonly covers a large area in order to decrease the water flow at the screen surface. This simple feature prevents newly hatched larvae from being damaged and from eventually clogging the screen. By using smaller mesh sizes (e.g. 150 μm), live prey, such as *Artemia* nauplii or enriched metanauplii, may be added and retained in the larval area. Since ornamental shrimp larvae commonly hatch during the night and are only collected and placed in larval rearing tanks at least by the next morning, the presence of live prey prevents newly hatched larvae from being exposed to starvation during this period. The assumption that yolk reserves of newly hatched larvae of all decapod species will be enough to satisfy their energy requirements for 12 or 24 hours of starvation is not correct. Unfortunately, this erroneous assumption is still widely accepted among researchers. Simões *et al.* (2002) have clearly documented how the starving of ornamental shrimp larvae immediately after hatching can negatively influence their quality and have an obvious adverse effect on the success of subsequent culture trials.

When stocking reproducing pairs of marine ornamental shrimps commonly associated with other invertebrates or fishes, it may not be necessary to also keep in captivity their host species (or symbiotic partners). Generally, host species are more demanding in terms of husbandry conditions, namely those displaying symbiotic zooxanthellae, which may necessitate the installation of more complex recirculated systems. Therefore, keeping shrimps with their symbiotic partners may be essential only when performing particular experimental trials to test settlement cues, host selection, costs and benefits of symbiotic behavior and symbiotic shrimp behavior during the moult cycle. As an example, partner shrimps of genus *Periclimenes* can be stocked without their host sea anemone and still mature in captivity (Calado, unpubl. obs.). Obviously, such 'unnatural' situations are only possible because reproductive pairs kept in maturation systems are not exposed to predation. Nevertheless, future studies should address the influence of symbiosis on the shrimp's dietary regimes and quantify the importance of host species in their nutrition (e.g. certain *Periclimenes* are known to eat the tips of the tentacles of their host sea anemone (Fautin *et al*., 1995)). In this way, researchers may decide on the importance of keeping symbiotic shrimps with their partners in captivity.

While forming breeding pairs of species displaying high levels of intraspecific aggression (e.g. *Stenopus* and pair-living *Lysmata*), a moveable plastic mesh (separating the breeding tank into two distinct areas) can also be used to physically separate them while chemical, tactile and visual contact between both specimens will still be possible. Through regular monitoring over a few days, it is possible to detect when agonistic behaviors (e.g. extended chelipeds) are no longer expressed, allowing shrimps to be safely placed together after establishing a pair bond. Empirical evidence seems to suggest that pairing aggressive species when the female shrimp has moulted, and is thus receptive to copulation, can reduce the risks of intraspecific aggression. Some ornamental shrimps living in large aggregations in the wild may also be kept in maturation tanks in small groups (e.g. *Rhychocinetes* and group-living *Lysmata*). In species with females displaying complex mating tactics, and positively selecting dominant males (e.g. *Rhynchocinetes*) (see Thiel & Hinojosa, 2003; Thiel & Correa, 2004; Van Son & Thiel, 2006), the best approach may be to keep a group of between two and five females with a single dominant male. It must be pointed out that the higher the number of individuals kept in the broodstock tank, the higher the chance of newly hatched larvae being cannibalized before being removed to the larval collector. In the case of hermaphroditic species of *Lysmata* living in groups, Zhang & Lin (2005c) have highlighted how small male-phase *Lysmata wurdemanni* could obtain more mating partners than larger conspecifics in the euhermaphrodite phase. Bauer (2005) has also pointed out that there is a cost of maleness for euhermaphrodite *Lysmata wurdemanni*, resulting in lower brood production for shrimps mating as male

and female when compared with a euhermaphrodite paired with a male-phase shrimp and mating exclusively as a female. None the less, it is obvious that a male–euhermaphrodite pair (as a whole) will always produce a lower number of larvae than a euhermaphrodite–euhermaphrodite pair. In this way, since the main commercial goal is to maximize the number of larvae produced using the lowest possible number of reproductive shrimps, euhermaphrodite–euhermaphrodite pairs should be used. Only if future studies reveal that the embryonic and/or larval quality of the offspring of male–euhermaphrodite pairs is significantly better than that of euhermaphrodite–euhermaphrodite pairs should they be used as broodstock for commercial purposes.

The need for only small sized maturation tanks makes it possible to set up inexpensive recirculated systems using small available areas, maximizing laboratory and commercial resources. Calado *et al.* (2007c) also highlight the importance of carefully choosing the materials used to provide shelter for breeding pairs in maturation tanks. Plastic mesh commonly used for fruit bags, or simply PVC pipes, can provide adequate shade, shelter and surface for shrimps to cling upside down (a position favoured by many ornamental decapods). Live rock is also a common choice, mainly because of its capacity to improve biological filtration and to provide the breeding pair with a more 'natural' environment. However, live rock also favours the accumulation of detritus, notably uneaten food, beneath it and increases the risks of introducing undesirable organisms into the system since these are common hitchhikers on live rock (namely amphipods, hydroids, glass anemones *Aiptasia* sp., and fire worms *Hermodice* sp.) (Nilsen & Fosså, 2002). Although some species may feed upon these organisms, they must always be regarded as potential predators of newly moulted shrimps or newly hatched larvae. The most threatening hitchhikers for ornamental shrimp recirculated systems are certainly hydroids. These organisms are commonly seen attached to imported live rock in their sessile and asexually reproducing life-stage, the polyp (Bolton & Graham, 2006). They rapidly establish large populations, and in extreme cases an entire batch of newly hatched larvae may be devoured in only a few hours (Calado, unpubl. obs.). In these situations the system must be shut down and filled with fresh water to permanently remove these pests. Obviously, this drastic measure also destroys the system's biological filter, which needs to be re-colonized by nitrifying and denitrifying bacteria. This can easily be regarded as one of most serious threats to the profitability of commercial ventures, since it delays ornamental shrimp production for weeks or even months. Another less serious drawback of employing live rock in maturation tanks is the fact that reproducing shrimps may feed on the organisms colonizing it when experimental trials on broodstock nutrition are being performed. If anything other than the experimental diet is being ingested by reproducing shrimps in an uncontrolled way, nutritional studies will always be inconclusive.

6.2 Choosing broodstock and triggering maturation in captivity

In order to successfully keep breeding pairs in captivity and induce their maturation, it is necessary to take into account the complexity of the different factors regulating these mechanisms. Small fluctuations in certain abiotic factors (such as water temperature and photoperiod), as well as insufficient or inadequate provision of nutritionally balanced dietary items, may impair the maturation of shrimps kept in captivity. Although sperm production and quality are also of utmost importance for reproductive success, the development of female gonads has always been studied in greater detail. Vitellogenesis in decapod crustaceans involves two distinct phases: primary and secondary vitellogenesis (Charniaux-Cotton, 1985). While primary vitellogenesis is a continuous process without seasonal variations, secondary vitellogenesis only occurs during the reproductive season. It is during secondary vitellogenesis that vitellogenin is taken up by oocytes. Vitellogenin is a lipoglycocarotenoprotein that has only been recorded in females (or simultaneous hermaphrodites) (Meusy, 1980). Secondary vitellogenesis is regulated by a complex interaction of two antagonistic hormones in decapod crustaceans: the gonad inhibiting hormone and the gonad stimulating hormone. The gonad inhibiting hormone is produced in the sinus gland located in the eyestalk (Panouse, 1943; Brown & Jones, 1949), while the gonad stimulating hormone is produced in the brain and thoracic ganglion (Otsu, 1963). Eyestalk ablation is now a commonly employed technique in decapod aquaculture, namely for penaied shrimps, since it plays a crucial role in inducing ovarian maturation in captivity. Basically, eyestalk ablation causes accelerated hormonal and metabolic changes, which stimulate ovarian maturation. The stage of ecdysis of ablated females, as well as their readiness to undergo gonadal development (e.g. females in a pre-vitellogenic state), can also condition the success of eyestalk ablation in triggering maturation. The nutritional status of females before ablation is vital for the success of this technique, since post-ablation dietary intake may not compensate for eventual imbalances present in broodstock that was unable to build up suitable nutrient reserves (Harrison, 1990). However, eyestalk ablation can also lead to a significant loss of embryonic quality and, in extreme situations, can even cause the death of ablated females (Benzie, 1998). Although this technique has already been successfully used in ornamental shrimps (e.g. in *Stenopus hispidus* by Zhang *et al*., 1997a), there is no need to employ this highly damaging technique to trigger maturation. Additionally, the reduced size of adult ornamental shrimp species, when compared with penaeid shrimps or lobsters, in association with their high market value, discourages the use of eyestalk ablation. Lin & Zhang (2001b) and Lin & Shi (2002) have been able to trigger ovarian maturation in marine ornamental decapods simply by ensuring good water quality, stable water temperatures in the range 26–28°C and a photoperiod of 12 hours of light and by providing broodstock with commercially available

diets. In the case of seasonal breeders (e.g in the wild *Lysmata seticaudata* only produces embryos during the warmer months of spring and summer (Dohrn, 1950; Couturier-Bhaud, 1974)), maturation can easily be triggered at any time of the year if water temperature is kept around 26°C, if suitable water quality is assured and if adequate dietary items are provided (Calado *et al*., 2007c). In certain situations maturation can still be triggered if water quality is not adequate, but several problems commonly occur:

(1) Reduced embryo production.
(2) Moults are discarded by the female still carrying numerous embryos attached to it.
(3) Larvae do not 'inflate' after hatching, remain curled and commonly die (Calado *et al*., 2005b). An initial warning signal of unsuitable water quality, which can be readily detected, is the presence of 'wrinkled' antennae in newly moulted shrimps.

When recruiting broodstock to form reproductive pairs, it is important to ensure that only high quality specimens are selected. A conscientious choice is only to prefer specimens that have been legally collected by fishermen employing sustainable practices. Another key aspect is to always quarantine any new shrimps before introducing them into maturation systems. Maturation in captivity will certainly be more readily triggered in healthy specimens than in shrimps which have been under any type of stress. In this way, avoid choosing as broodstock, shrimps with the following characteristics.

(1) Missing or displaying damaged pereiopods or antennae. Although these appendages can be easily regenerated in following moults, it is possible that pathogenic organisms may have colonized the damaged body parts and may present a threat to other specimens.
(2) Black gills, since these are commonly present in shrimps which have been kept in systems with poor water quality favouring gill fouling by organic matter and microorganisms. In many cases, ornamental shrimps displaying black gills completely recover a normal appearance after moulting (Calado, unpubl. obs.).
(3) Exhibiting unusual bumps in the lateral regions of the carapace or in the ventral area of the abdomen. These bumps are commonly caused by the presence of parasitic bopyrid isopods. Bopyrid isopods are holoparasites, which have decapods as their final hosts (Markham, 1986). The life history of these parasites can be briefly described by the existence of a free-swimming epicaridean larva that attaches itself to a calanoid copepod, acting as an intermediate host. The larva metamorphoses into a microniscus (Dale & Anderson, 1982) and remains in the copepod until it metamorphoses again to a free-swimming cryptoniscus larva. The cryptoniscus eventually leaves the copepod and infects the final host, with

the first cryptoniscus that settles metamorphosing into a female. Future cryptonisci settling on the same host will always metamorphose into males (O'Brien & Van Wyk, 1985). Usually, a final host will have a single female bopyrid in the branchial chamber or clinging to its abdomen, accompanied by one or two dwarf males (Anderson, 1990). Bopyrid isopods cause the parasitic castration of their hosts, impairing the maturation of the female's gonads or feminizing parasitized males (O'Brien & Van Wyk, 1985). The host reproductive potential is always substantially reduced and in gonochoric species 'reproductive death' can be recorded (Van Wyk, 1982). Like gonochoric shrimps, hermaphroditic species of genus *Lysmata* parasitized by bopyrids also display a lower reproductive potential. However, the complete 'reproductive death' of the host does not occur. Bopyrid isopods infecting the abdomen of *Lysmata* in the euhermaphrodite phase caused only the parasitic castration of the female function (Fiedler, 2000; Calado *et al*., 2005c). The branchial bopyrid isopod *Parabopyrella* sp. significantly affects the female sexual system of *Lysmata amboinensis*, reducing the number of produced embryos, but does not cause parasitic castration (Calado *et al*., 2006). Although Fiedler (2000) reported the infection by bopyrid isopods of some shrimps kept in captivity, mass infections of broodstock will not be a common situation since the intermediate host copepod must be present in the recirculated water.

6.3 Broodstock nutrition

Scientific studies addressing the nutritional requirements of marine ornamental shrimps are still insufficient, and the identification, as well as quantification, of most essential dietary requirements has not yet been made. It is well known that diets provided to broodstock in captivity significantly affect the quality of gametes embryos and larvae (Harrison, 1990). Most studies addressing dietary requirements in decapod crustaceans are biased towards the species having a higher market value for human consumption. The abundant information available in this field for penaeid shrimp (Wouters *et al*., 2001) may provide valuable help for researchers addressing the dietary requirements of ornamental shrimps. However, it must be highlighted that because of their taxonomic distance, expressed in different reproductive behavior (penaeids release their embryos into the water column whereas caridean ornamental shrimps incubate them under their abdomen), some differences may exist between the dietary requirements and metabolic pathways of these two groups of decapods. Studies addressing the nutrition of clawed and spiny lobsters (e.g. Gendron *et al*., 2001; Smith *et al*., 2004) may also be helpful for ornamental shrimps. Unfortunately, the only group of caridean shrimps that have had their nutritional requirements widely studied is the freshwater species

in the genus *Macrobrachium* Bate, 1868 (e.g. Cavalli *et al*., 2000a; Gupta *et al*., 2007). Researchers working with ornamental shrimps and employing available information on *Macrobrachium* spp. nutrition must remember that nutritional requirements for freshwater and marine shrimps may present more or less significant differences (D'Abramo, 1998). Despite such differences, the experimental methodologies and global nutritional requirements already known for decapod crustaceans must be carefully considered by researchers working with marine ornamental shrimps.

Another important constraint when trying to ascertain the dietary requirements of marine ornamental shrimps is the lack of information on their reproductive performance or feeding habits in the wild. By understanding embryo production in wild populations of marine ornamental shrimps, it may be possible to determine the expected size of larval batches of shrimps maturing in captivity. In these studies it is important to accurately record shrimp size, as well as the developmental stage of embryos being incubated, since these two aspects significantly affect egg production (Calado & Narciso, 2003a). This quantitative approach allows researchers to quickly identify the suitability of maturation diets simply by comparing the number of embryos being produced in captivity with those that would be produced in the wild by a shrimp of a similar size. Studying the natural feeding regimes of marine ornamental shrimps can be vital for the development of suitable diets for shrimps maturing in captivity. However, this is far from being an easy task since absolute or relative amounts of food ingested are not easy to quantify. Additionally, identification of dietary food items is sometimes impossible because of the highly effective mouthparts of decapods and their gastric mill ossicles, which reduce food to very small fragments (Williams, 1981).

Commonly, two different methods are employed to study gut content in decapods: the points method and the occurrence method. The points method is an approximate volume measure and is inaccurate for monitoring soft food items since they are rapidly processed and quickly become unrecognizable. This method is more suitable for food items ingested in large, recognizable pieces. The occurrence method measures the regularity of inclusion of a particular food item in the diet, although it is not suitable for foods with no recognizable hard parts (Williams, 1981). Despite the method followed when analysing decapod gut content, important food items are always distinguished from rare and unimportant ones. Becker & Grutter (2004) could easily recognize the presence of crustacean ectoparasites infesting fishes in the guts of *Urocaridella* sp. and *Periclimenes holthuisi*. For commercial purposes, it is highly important to understand the relevance of ectoparasites in cleaner shrimps' diets. Do these prey contribute any specific nutrients? Can they adequately be replaced in captivity by other dietary items? The same rationale can be applied to other ornamental shrimp species displaying particular dietary habits. Do partner shrimps, clipping the tips of the tentacles of their host sea anemones (Fautin *et al*., 1995), seek the proteins of the tentacles or the

carbohydrates being produced by the symbiotic zooxanthellae? Do harlequin shrimps require specific nutrients from sea stars? And the most pertinent of all interrogations: can we mimic all dietary requirements in captivity?

The biochemical composition of developing embryos can also provide valuable guidelines for the formulation of suitable maturation diets (Rosa *et al*., 2005). Through the analysis of the biochemical composition of early developing embryos, it is possible to predict which reserve substances are being mobilized by females during vitellogenesis (Rosa *et al*., 2003). By knowing which substances are mobilized during vitellogenesis, researchers can significantly improve broodstock condition by supplying those nutrients through tailor-made maturation diets (Chen, 1998; Teshima, 1998). Despite the high potential of this experimental approach, so far the only data available for marine ornamental shrimps is that for *Lysmata seticaudata* (Calado *et al*., 2005d). Although the study only addressed amino acid and fatty acid dynamics during embryonic development, it clearly shows how intraspecific and seasonal variations may affect the biochemical profile of early developing embryos. Werhtmann & Graeve (1998) and Werhtmann & Kattner (1998) also highlighted the importance that annual and geographical variations may have for the composition of egg yolk reserves of developing decapod embryos. In this way, studies addressing the biochemical composition of marine ornamental shrimp embryos should consider these variations, with recorded values being used as qualitative, rather than quantitative, guidelines.

So far, the studies addressing the nutritional aspects of maturation in marine ornamental shrimps have mostly relied on a variety of available fresh and/or frozen foods: enriched and regular *Artemia* nauplii and adult biomass, clam, mussel, krill, shrimp, squid, polychaetes and commercial mixed diets (e.g. Marine Cuisine® produced by San Francisco Bay Brand®) (Simões *et al*., 1998; Lin & Zhang, 2001b; Lin & Shi, 2002; Calado *et al*., 2007c). Crustacean tissues have been a key component for most maturation diets for decapods (e.g. Cavalli *et al*., 1997; Crocos & Coman, 1997) since their inclusion provides a nutritional source to replace the wide variety of crustacean prey available for broodstock in the wild (Rothlisberg, 1998). Wouters *et al*. (2001) also note that tissue of sexually mature crustaceans used in maturation diets can be a valuable source of reproductive hormones, contributing to endocrinological pathways related to gonadal maturation. Despite these potential benefits, the risks of disease transmission, by feeding crustacean tissues to broodstock, is now considered as too high for enterprises addressing biosecure broodstock production, particularly of penaeid shrimps (Wouters *et al*., 2001). Frozen and fresh food items rapidly decay after being supplied and quickly lead to deteriorating water quality (Sheen & Wu, 1999). Despite the growing interest in commercial-scale culture of marine ornamental shrimps, there are still no specific formulated diets available in the market for these organisms (Lin *et al*., 2002). Artificial diets are vital for experimental studies addressing exact

nutrient requirements of broodstock shrimps maturing in captivity (Djunaidah *et al*., 2003).

In shrimp culture, formulated diets are commonly used in the form of pellets and have the following main advantages over fresh or frozen diets (Harrison, 1990):

(1) regular availability;
(2) reproducible and controlled composition and quality;
(3) ease of dosing;
(4) improved stability under storage;
(5) reduced tank fouling;
(6) reduced risks of pathogen introduction in culture systems; and
(7) higher versatility for the supplementation of therapeutics, immunostimulants and hormones.

Feeding regimes using a combination of several dietary items, namely formulated diets and fresh foods, commonly outperform single-item fresh or frozen diets (Bray *et al*., 1990; Nascimento *et al*., 1991; Calado *et al*., 2007c). Nevertheless, most commercial facilities culturing decapods replace only small proportions of fresh or frozen dietary items by pellets, using formulated diets more as a supplement to broodstock. This cautious approach is followed to reduce the risk that reproducing broodstock might not receive the nutrients required for maturation, since, even for widely studied penaeid shrimps, there is still limited knowledge on the nutrient requirements of adult shrimps during maturation (Wouters *et al*., 2001). As pointed out by Glencross *et al*. (2007), 'a feed is only as good as its ingredients', an aspect of vital importance for the formulation of suitable maturation diets. The need to use premium ingredients for manufacturing diets to serve a somewhat limited market (in comparison with other fields of animal production) makes these artificial diets expensive (Wouters *et al*., 2001). Nevertheless, this may not be a limiting factor for ornamental shrimp culture, mainly because of the high market values these organisms may achieve in the aquarium trade.

When maturing broodstock initiates the energetic bioaccumulation necessary to undergo gonadal maturation, endogenous nutrient reserves are rapidly mobilized and need to be properly replenished. Assuring the replenishment of mobilized reserves is particularly important for compounds that cannot be synthesized *de novo* by broodstock and need to be provided by the maturation diet (Harrison, 1990). The composition of suitable maturation diets (fresh, frozen or formulated) must always consider the following nutrients before they can be successfully employed in broodstock maturation in captivity:

- lipids (total, fatty acids and classes)
- proteins
- carbohydrates

- vitamins
- minerals
- carotenoids

6.3.1 Lipids

Shrimps do not seem to have an absolute requirement for dietary lipids, rather displaying specific requirements for certain nutrients, namely highly unsaturated fatty acids (HUFA), phospholipids and sterols (D'Abramo, 1989). These specific requirements are mainly relevant concerning HUFA, owing to shrimps' reduced ability to synthesize them *de novo* (Mourente, 1996), and sterols, since shrimps are unable to carry out *de novo* synthesis of the steroid ring (Teshima & Kanazawa, 1971; Kanazawa *et al*., 1988). Total lipid levels in some shrimp broodstock diets may reach 14% or even more (Wouters *et al*., 2001). However, high dietary lipid levels may negatively affect consumption rate, since shrimps may rapidly get satiated when their energetic requirements are satisfied. This imbalance in consumption rates may involuntarily cause nutritional deficiencies in maturing broodstock (D'Abramo, 1997). The hepatopancreas, also known as the digestive gland, is the major lipid storage and processing organ in decapod crustaceans (Vogt *et al*., 1985). This organ may transfer a significant proportion of lipids to developing ovaries via the hemolymph (Teshima *et al*., 1988a, b). Consequently, while total lipid concentrations suffer a marked increase during maturation, the hepatopancreas suffers a marked decrease in its lipid content. None the less, in certain decapods, a major proportion of lipids accumulated in the ovaries seem to originate from dietary items (Castille & Lawrence, 1989) and not from lipid reserves stored in the hepatopancreas. After verifying a low level of ovarian *de novo* lipid synthesis and the presence of insufficient lipid reserves in the hepatopancreas, Clarke (1982) suggested that, in some species, this organ may just modify incoming lipids from dietary items, which will later be exported to the ovaries during maturation.

Highly unsaturated fatty acids, namely those of the *n*-3 (formerly known as *omega*-3) group, seem to occur at a higher proportion in ovarian lipids than in the hepatopancreas. Among *n*-3 HUFA, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (20:5*n*-3 and 22:6*n*-3, respectively) are considered of major relevance, with the first playing a significant role in ovarian development and the second in early embryogenesis (Cahu *et al*., 1994, 1995; Xu *et al.* 1994). The concern of producers to provide these nutrients in broodstock diets is commonly addressed by providing natural food organisms, such as squid and bloodworms (Middleditch *et al*., 1980; Lytle *et al*., 1990; Coman *et al*., 2007), or enriched adult *Artemia* in *n*-3 (Naessens *et al*., 1997; Wouters *et al*., 1999; Lin & Zhang, 2001b; Lin & Shi, 2002), containing high levels of *n*-3 HUFA. Marine shrimps have a very limited ability to elongate and desaturate polyunsaturated

fatty acids (PUFA), in order to produce HUFA (Kanazawa *et al*., 1979a, b), with EPA and DHA being considered essential fatty acids. Arachidonic acid (ArA, 20:4*n*-6) is also an important HUFA for marine shrimps, being a precursor in the synthesis of prostaglandins, which play an important role in the reproductive processes of crustaceans (Harrison, 1990). Because of the complexity of these metabolic pathways, it is almost impossible to predict the amount of HUFA that may be synthesized by broodstock fed a PUFA-enriched diet. Therefore, it is advisable to provide C20 and C22 HUFA rather than their C18 precursors. None the less, the PUFA linolenic acid (LNA) and linoleic acid (LOA) (18:3*n*-3 and 18:2*n*-6, respectively) are also advocated as essential fatty acids (Glencross & Smith, 1999), which need to be properly balanced in broodstock diets. There is a generalized belief that a high *n*-3/*n*-6 ratio should be present in maturation diets (Lytle *et al*., 1990), supporting the inclusion of high levels of EPA and DHA and moderate levels of ArA. Nevertheless, the accurate balance between these two groups of fatty acids is still not known, nor are the interactions of different amounts of essential fatty acids in the performance of maturation diets, namely their digestibility (Glencross & Smith, 1999, 2001; Glencross *et al*., 2002).

Triacylglycerides (TAG), phospholipids (PL) and cholesterol are considered the major lipid classes present in mature ovaries (Wouters *et al*., 2001). Triacylglycerides are neutral lipids, being a major source of energy and the predominant form of energy storage in shrimps (Clarke, 1982). During maturation, there is a remarkable increase in TAG levels in ovaries and a sharp decrease in spent ovaries (Ravid *et al*., 1999). It appears that TAG are selectively incorporated in embryonic reserves and play an important role as energy sources during embryogenesis. Palacios *et al.* (1998, 1999) also found significant evidence indicating that reproductive exhaustion can be related to a deficiency in the transfer of TAG to the offspring.

Phospholipids have important functions as cytoplasm and membrane constituents of cells, and are therefore responsible for several structural and physiological properties (Harrison, 1990). They also function as a source of choline, inositol, essential fatty acids and energy, while improving diet quality by increasing its stability and palatability (Coutteau *et al*., 1997). In general, lipids are transported in the haemolymph in the form of PL, in a protein to lipid ratio of 1:1 to 1.2:1 (Lee & Puppione, 1978, 1988). They are present in high quantities in shrimp ovaries, mainly as phosphatidylcholine and phosphatidylethanolamine (Ravid *et al*., 1999). Shrimp broodstock has a dietary requirement for PL (Bray *et al*., 1990; Alava *et al*., 1993a) since crustaceans have a limited ability to biosynthesize PL *de novo* (Shieh, 1969). Cahu *et al.* (1994) suggested that broodstock diets should contain more than 2% phospholipids, in order to ensure that 50% of total egg lipids are represented by phospholipids.

Cholesterol is also an important component in all cell membranes (Harrison, 1990). Kanazawa & Teshima (1971) have demonstrated the *in vivo* conversion

of cholesterol to steroid hormones in ovaries of spiny lobsters. Since decapod crustaceans are unable to perform *de novo* synthesis of the steroid ring (Zandee, 1967), cholesterol derived from dietary items is stored in the muscle, hepatopancreas and gonads (Middleditch *et al.*, 1980). While other sterols may partly replace the dietary requirements for cholesterol, it is possible that their bioconversion may not be efficient enough to provide suitable levels of cholesterol to developing oocytes (Harrison, 1990). Therefore, cholesterol is commonly considered essential and is usually provided in maturation diets (Kanazawa *et al.*, 1988). One of the main reasons for producers to provide clam or squid to maturing broodstock is their cholesterol content. The increase in ovarian sterols during maturation, and the concomitant decrease in hepatopancreas cholesterol, suggest that stored reserves are mobilized to allow the ovarian build-up of cholesterol levels (Zandee & Kruitwagen, 1975; Teshima & Kanazawa, 1983a; Lautier & Lagarrigue, 1988). However, Kanazawa *et al.* (1988) recorded experimental evidence indicating that cholesterol is mobilized to the ovaries from reserves located in the muscle. In this way, while the hepatopancreas may be the major site for cholesterol metabolism, cholesterol being mobilized and sequestered in the ovaries during maturation originates from the muscle.

6.3.2 Proteins

Given the occurrence of intense biosynthesis during maturation, it is assumed that protein requirements play a higher role than in non-reproductive periods (Harrison, 1990). Dietary proteins provide essential and non-essential amino acids for muscle, connective tissue and haemolymph respiratory protein manufacture. The synthesis of ovarian and haemolymph lipoproteins (e.g. vitellin and vitellogenin, respectively) is of major importance during maturation. Vitellogenin plays a significant role in the transport of lipids since they are insoluble in water and only after binding with lipoproteins can they be transported in the haemolymph (Harrison, 1990). Formulated diets commonly have about 50% protein, although this proportion is lower than that displayed by fresh or frozen diets (Harrison, 1990). Since optimal dietary protein levels depend on protein source and are species specific, it seems to be more pertinent to address amino acid requirements, namely those which are considered as essential for shrimps (threonine, methionine, valine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine and tryptophan) (Cowey & Forster, 1971; Shewbart *et al.*, 1972; Wouters *et al.*, 2001). A common recommendation when formulating artificial diets is to mimic the amino acid profiles present in fresh dietary items (Deshimaru, 1982) since there is still a lack of experimental studies addressing optimal protein levels, energy ratios and amino acid profiles for shrimp broodstock diets (Wouters *et al.*, 2001).

6.3.3 Carbohydrates

Dietary carbohydrates are not considered essential for broodstock, although crustaceans exhibit a strong ability to catabolize them in comparison with other marine invertebrates (Kristensen, 1972). Carbohydrate metabolism appears to be species specific in decapods, with dietary monosaccharides being rapidly absorbed but poorly used. In some species monosaccharides may even induce hyperglycaemia and suppress growth (Lim & Persyn, 1989). Radford *et al.* (2005) suggest that hyperglycaemic responses induced by monosaccharides may be due to the bypassing of contact digestion and absorption in the R-cells of the hepatopancreas or by monosaccharides directly eliciting the release of crustacean hyperglycaemic hormone via a chemosensory reflex. In contrast, disaccharides and polysaccharides seem to be more effectively used by decapods. This can probably be explained by polysaccharides not being absorbed in the stomach, since they are converted to monosaccharides in the midgut and hepatopancreas and only then are they gradually released into the haemolymph (Deshimaru & Yone, 1978; Abdel-Rahman *et al.*, 1979). Carbohydrates are commonly stored as glycogen in the muscle and hepatopancreas and may play a significant role in the production of ovarian pigments (carotenoglycolipoproteins). They are also important in the production of glucosamine, a precursor of chitin (the principal constituent of the exoskeleton of decapods) (Harrison, 1990). During maturation, a significant increase in glycogen levels in the ovaries and testes is accompanied by a simultaneous depletion of this compound in the hepatopancreas, suggesting mobilization (Kulkarni & Nagabhushanam, 1979; Nagabhushanam & Kulkarni, 1981). In order to spare expensive protein in formulated diets, starch is commonly included as a low-cost bulk-energy component (Glass & Stark, 1995). However, a more cautious approach should be used when addressing diet formulation for broodstock, since the intensity of biosynthetic activity during maturation may indeed require high levels of protein, thus minimizing the chances of replacing expensive dietary protein by low-cost carbohydrates (Harrison, 1990).

6.3.4 Vitamins

Vitamin requirements for decapod crustaceans during larval culture and early juvenile growth have already been addressed in several studies (e.g. Conklin, 1997; D'Abramo, 1997; Merchie *et al.*, 1997; Reddy *et al.*, 1999). However, information on the dietary requirements for vitamins during maturation is still scarce, and is sometimes extrapolated from other marine invertebrates and fishes. None the less, it is expected that during maturation, a period of intensive biosynthesis, vitamin mobilization to developing oocytes and sperm may occur. Vitamins are divided into two distinct groups: fat-soluble [e.g. retinol (A), cholecalciferol (D), α-tocopherol (E) and menadione (K)] and water-soluble

[e.g. thiamine (B_1), pantothenic acid, riboflavin (B_2), inositol, pyridoxine (B_6), choline, folic acid, niacin, cyanocobalamine (B_{12}), biotin and ascorbic acid (C)] (Harrison, 1990). Vitamins are commonly supplied in broodstock artificial diets through complete vitamin premixes. The recommended levels are primarily based on empirical evidence rather than on systematic experiments. It is known that vitamin levels may be significantly affected by heat during the processing of artificial diets, as well as by leaching after they are provided to broodstock and immersed in sea water (Gadient & Schai, 1994). A common strategy to compensate for these losses is to over-fortify commercial diets in these particular nutrients, ensuring that vitamins are effectively available to broodstock (Harrison, 1990).

Perez-Velazquez *et al.* (2003) verified how the supplementation of maturation diets with vitamins significantly increased the performance of male penaeid shrimps (concerning sperm production). These authors highlighted how the traditional combinations of fresh-food organisms commonly used in captivity are far from nutritionally balanced. A positive effect on fecundity and larval quality was recorded by Mengqing *et al.* (2004) when providing higher levels (60 mg/kg) of vitamin A to penaeid broodstock. Pangantihon-Kühlmann *et al.* (1998) also found that vitamin A supplementation of maturation diets highly enhanced ovarian development and spawning.

The importance of ascorbic acid (vitamin C) in broodstock diets must also be stressed since crustaceans, unlike most animals which can synthesize this vitamin from glucuronic acid, lack the enzyme gulonolactone oxidase necessary for the last step of ascorbic acid biosynthesis (Chatterjee, 1973). Consequently, broodstock shrimps depend on the constant supply of adequate levels of vitamin C provided through maturation diets (Merchie *et al.*, 1997). Cahu *et al.* (1994) reported that dietary levels of vitamin C, as well as being an important antioxidant, directly affected the content of this nutrient in penaeid embryos, with higher contents apparently inducing higher hatching rates. A delay on ovarian maturation was also reported by Alava *et al.* (1993b) for penaeid broodstock fed diets with deficient levels of vitamin A, E and C. None the less, several questions concerning the importance of vitamin levels in shrimp maturation diets still need to be properly addressed, namely:

(1) What is the qualitative and quantitative increment in vitamin requirement during maturation?
(2) Are those increments similar for males and females?
(3) Are there any toxic effects due to hypervitaminosis?
(4) What are the interactions between vitamins and other nutrients (e.g. carotenoids and minerals)?
(5) Are all vitamins equally important (fat-soluble and water-soluble)?
(6) Are there any species-specific requirements or is there a common ecological and/or phylogenetic trend?

6.3.5 Minerals

The evaluation of mineral requirements in marine crustaceans is complicated by their ability to absorb these nutrients from surrounding water, namely across their gill and intestinal cells (Harrison, 1990). In certain situations it may therefore be almost impossible to distinguish mineral dietary requirements from physiological ones. In order to allow a better understanding of mineral requirements during experimental trials, data on the levels of minerals present in the diet (including those in dietary items and mineral mixes) should be provided along with those of waterborne minerals. Although shrimp mineral requirements during maturation are still poorly understood, Harrison (1990) pointed out that mineral deficiencies, particularly of macroelements, may negatively affect reproduction in two ways: (1) by inducing physiological stress, reducing the reproductive fitness of broodstock, namely by significantly changing their metabolism (e.g. respiration and excretion); and (2) by altering oocyte (and consequently embryonic) mineral composition, negatively affecting hatchability and larval viability. As for vitamins, these nutrients are also supplemented as premixes in artificially formulated diets (Wouters *et al*., 2001) with the following minerals deserving special attention: (1) macrominerals — calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl); and (2) microminerals — iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se) and cobalt (Co). In the aquarium hobby, iodine (I) and strontium (Sr) supplementation of sea water, mainly through commercial additives, has gained popularity in recent years, and is even considered vital for keeping ornamental shrimps successfully in captivity. However, there are still no studies supporting or rejecting the importance of supplementing these minerals, as either waterborne or dietary nutrients. Mineral premixes used in penaeid shrimp artificial diets commonly fortify Ca, P, Mg, Na, Fe and Se levels (Marsden *et al*., 1997; Wouters *et al*., 2001), although negative consequences for bioavailability and mineral metabolism may occur in diets with high ash (total minerals) content. The study by Davis *et al.* (1992) seemed to indicate the dietary essentiality of Mg, Mn, Fe, Zn and Cu, and the authors also suggested that Ca and P levels may also be significantly affected by dietary levels. As for many other nutrients, mineral dietary requirements for shrimp maturation diets will only be clarified after performing elemental analysis on wild broodstock with developing gonads, embryos and newly hatched larvae.

6.3.6 Carotenoids

The dietary importance of carotenoids has been recognized in pigmentation, as a source of provitamin A, in antioxidant functions, in cellular protection from photodynamic damage, in growth enhancement and in reproductive potential (Liñán-Cabello *et al*., 2002, 2003). Carotenoids have also been proposed to

stimulate the immune system and enhance gonadal maturation and embryonic development (e.g. pigmentation of the egg cuticle, chromatophores and eyespots) (Petit *et al*., 1991; Olson, 1993; Dall, 1995; Mantiri *et al*., 1995; Liñán-Cabello *et al*., 2004). These compounds are also known to act as antioxidants and affect fertilization success (Olson & Owens, 1998), commonly being used in artificial diets to prevent peroxidation of PUFA (Wouters *et al*., 2001).

Crustaceans are incapable of *de novo* synthesis of carotenoids, being totally dependent on dietary sources (Harrison, 1990). Deficiency of dietary carotenoids is associated with a loss of these nutrients in the ovaries of mature females, as well as in the egg yolk, and promotes disruptive effects in the endocrine system regulating gonadal development and maturation (Gilchrist & Lee, 1972; Meyers, 1994). Dietary carotenoids are also the sole biological precursors of retinoids in crustaceans. Retinoids are bioactive molecules that are involved in the activation of hormonal nuclear receptors and play an important role in embryonic development and cell differentiation (Liñán-Cabello *et al*., 2002). However, the inclusion of retinoids in artificial maturation diets appears to be unnecessary as long as adequate levels of carotenoids are supplied (Dall, 1995).

Astaxanthin and its esters have been reported as the dominant carotenoid in shrimp ovaries and hepatopancreas (Dall *et al*., 1995; Petit *et al*., 1997), followed by zeaxanthin and β-carotene (Liñán-Cabello *et al*., 2002). Their relative proportions in these organs differ markedly during maturation, reflecting the complex nature of the metabolic pathways taking place during this period and their mobilization from the hepatopancreas to the ovaries via the haemolymph (Vincent *et al.* 1988a, b; Petit *et al*., 1997; Pangantihon-Kuhlmann *et al*., 1998).

Several approaches to the supplementation of maturation diets with pure carotenoids, or carotenoid-containing compounds, have already been tested (Liñan-Cabello *et al*., 2002). Wyban *et al.* (1997) showed the potential use of food-grade paprika to supplement maturation diets, since broodstock appears capable of transforming α-carotene, α-cryptoxanthin, capxanthin and capsorubin into astaxanthin. The supplementation of *Spirulina* in maturation diets has also been successfully used to correct carotenoid deficiency in broodstock shrimp diets (Regunathan & Wesley, 2006). The authors report that *Spirulina* supplied the required carotenoids to shrimps undergoing maturation, boosting the quantity of total carotenoids in the ovary, which resulted in an improved carotenoid content of developing embryos. None the less, it is still necessary to clearly identify the interactions between carotenoids and other nutrients present in maturation diets, namely vitamins (Pangantihon-Kuhlmann *et al*., 1998). Additionally, there is a need to investigate the most suitable sources of dietary carotenoids that should be included in maturation diets, in order to prevent lower reproductive performances of broodstock kept in captivity after several spawning events (Wyban *et al*., 1997; Palacios *et al*., 1999).

6.4 Broodstock condition and larval quality

Continuous larval production in captivity is vital for the establishment of commercial-scale culture of marine ornamental shrimps. Broodstock shrimp maturation in captivity directly affects larval production, both quantitatively and qualitatively. Commonly, higher reproductive performances are recorded in shrimps collected from the wild that already display mature gonads, probably as a consequence of a higher uptake of key nutrients required for metabolic pathways related to maturation (e.g. vitellogenesis) in their natural environment. In captivity, reproductive shrimps are totally dependent on the nutrients provided by maturation diets to fulfil the high nutritional requirements related to reproduction, with any dietary deficiencies or imbalances being reflected in lower reproductive performance (Palacios *et al*., 1999). The size of broodstock females quantitatively affects larval production, since caridean shrimps commonly display an isometric relation between body and brood size (Bauer, 1991). Therefore, as long as resulting offspring quality is not affected, the selection of the largest individuals for broodstock should be preferred. However, shrimp size and age are closely related and age may influence reproductive performance and offspring quality (Crocos & Coman, 1997). Cavalli *et al.* (1997) and Rothlisberg (1998) highlight the possibility of senescence occurring in larger (thus older) shrimp, negatively affecting their reproductive performance. Although the studies on this topic are practically non-existent for marine ornamental shrimps, Calado & Narciso (2003a) and Calado *et al.* (2005d) did not find any evidence of senescence in *Lysmata seticaudata* embryo production or biochemical composition of developing embryos. Nevertheless, it is possible that senescence may occur in certain marine ornamental shrimp genera or species, meaning that larger may not mean better broodstock. Exhaustion of broodstock is also an important aspect of the management of reproductive shrimp pairs, reflecting the decline in their reproductive performance under intensive maturation conditions. This decline commonly depends on several conditions affecting broodstock in captivity, namely maturation diet, broodstock origin, environmental factors and genetic variability (Racotta *et al*., 2003). Penaeid shrimp broodstock is commonly replaced approximately 3 months after being kept in captivity, although this period can be extended for unablated shrimp (Browdy & Samocha, 1985) or through nutritional improvements to maturation diets (Marsden *et al*., 1997; Wyban *et al*., 1997). Because of the high market value of marine ornamental shrimps, periodic replacement of reproductive pairs may be an economic burden for commercial-scale culture. The performance of newly collected wild shrimps is commonly superior to that of wild specimens kept in captivity for more than 6 months or when using captive cultured shrimps as broodstock (Calado, unpubl. obs.). However, captive-cultured specimens may also display high reproductive performance (Coman & Crocos, 2003; Peixoto *et al*., 2003) and simply excluding these organisms

from broodstock may prove to be an erroneous approach. None the less, the periodic replacement of broodstock seems inevitable in order to ensure feasible larval production in quantity as well as in quality. Since the highly damaging technique of eyestalk ablation is not required to trigger maturation in marine ornamental shrimps, it is possible that future improvements concerning maturation diet formulation may allow broodstock to achieve high reproductive performance for longer periods. This approach will significantly reduce the periodic need to replace exhausted broodstock. Genetic variability may also affect broodstock quality, with certain reproductive pairs displaying higher performance than others and producing high quality larvae. Although genetic studies addressing reproductive performance of penaeid shrimps are already being developed (see Ibarra *et al*., 2007, for a review), there is still no available information for marine ornamental shrimps. A first approach for genetic improvement of marine ornamental shrimp broodstock may be the selective breeding of specimens displaying high reproductive performance and selecting some of their offspring for broodstock. However, this will only be possible if inbreeding is avoided (through rigorous tracking of offspring pedigree) and if maturation diets can fully ensure that all nutritional requirements associated with reproduction are met. According to Racotta *et al*. (2003), the term 'larval quality' is commonly employed to address:

(1) larval physiological condition;
(2) the performance of larvae during culture trials; and
(3) their stress resistance to physical manipulation, variable culture conditions (e.g. salinity and temperature) or pathogens.

The following criteria are commonly proposed for the evaluation of larval quality: biochemical, morphological, behavioral, productive and survival to stress tests (Racotta *et al*., 2003).

6.4.1 Biochemical criteria

The biochemical composition of larvae and eggs is used as a predictive criterion of their quality. Because of their importance as energetic reserves, as well as their structural role, lipids can be analysed to estimate larval quality since these nutrients are gradually consumed during embryonic and larval development (Palacios *et al*., 2001a). Analysis of total lipids, triglycerides, phospholipids and/or HUFA variations has been successfully used to estimate shrimp larval quality (Coutteau *et al*., 1997; Cavalli *et al*., 1999; Palacios *et al*., 1999, 2001b).

Although several studies have already addressed the levels of vitellin in developing embryos and early larvae (Quackenbush, 1989), it is still not clear if this can be used as a reliable indicator of larval quality. If future studies confirm that the analysis of vitellin levels can effectively be used to ascertain

larval quality, the lipid composition of the vitellin must certainly be taken into account (Racotta *et al.*, 2003).

Protein content is also highly significant in decapod embryos and newly hatched larvae, playing structural and energetic roles, although no relation to larval quality has yet been detected (Lemos & Rodriguez, 1998; Palacios *et al.*, 1999). Nevertheless, it is possible that digestive enzyme activity of newly hatched larvae may be successfully used to determine larval quality (Jones *et al.*, 1997a).

The levels of carotenoids, namely astaxanthin, in embryos and larvae have also been used to validate larval quality, with batches that display higher contents of these nutrients exhibiting higher survival (Wyban *et al.*, 1997; Palacios *et al.*, 1999). This higher performance of embryos and larvae with higher levels of carotenoids may be due to their important role as natural antioxidants against other biochemical components and light, which seems to be vital for the protection of HUFA (Wouters *et al.*, 2001).

Carbohydrates are rarely used as a criterion to assess larval quality, although they must play an important role in the synthesis of chitin during larval development and consequently influence larval performance during the moult cycle (Racotta *et al.*, 2003).

So far, the analysis of mineral and vitamin levels as indicators of larval quality has had little attention, although these nutrients are highly involved in the endocrine processes regulating larval moulting cycles.

The RNA/DNA ratio as an index of growth has been successfully used in several marine invertebrates (Wagner *et al.*, 1998; Vidal *et al.*, 2006). The rationale for the use of this ratio is that since cellular RNA is essential for the biosynthesis of proteins, the amount of RNA must increase rapidly during growth while the cellular DNA content remains constant. In this way, the RNA/DNA ratio reflects recent growth, being an index of the cell's synthetic capacity (Clemmesen, 1994). However, the suitability of the RNA/DNA ratio to determine larval quality of crustacean larvae still needs to be evaluated.

6.4.2 Morphological criteria

Size and weight have been the main features used to evaluate the larval quality of marine shrimps, although color, the presence of deformities, digestive system morphology and the presence of fungi, bacteria and viruses are occasionally also used (Racotta *et al.*, 2003). Several studies have already recorded the existence of significant correlations between egg volume and different aspects of yolk content in shrimps (Clarke, 1993; Rosa *et al.*, 2007). However, there is still no experimental evidence relating egg size with larval quality (Palacios *et al.*, 1998, 1999). Since caridean shrimps already hatch in the zoeal stage, the naupliar condition index proposed by Palacios *et al.* (1998, 1999) for penaeids appears to be inadequate for marine ornamental shrimps.

Bray *et al.* (1990) have proposed zoeal length as an index of larval quality for broodstock nutrition studies. However, although some studies were able to relate higher zoeal lengths to higher larval survival (Wouters *et al.*, 1999), it seems that this is not a totally reliable criterion, with similar studies failing to obtain such trends (Hernández-Herrera, 2001). Therefore, the interpretation of morphological criteria to ascertain larval quality must be cautious, since intra- and interspecific variability seems to occur.

6.4.3 Behavioral criteria

The positive phototactism displayed by newly hatched decapod larvae has been commonly used to facilitate their harvest from recirculated systems, as well as being a predictive indicator of larval quality (Smith *et al.*, 1993; Treece & Fox, 1993; Calado *et al.*, 2007c). Ibarra *et al.* (1998) confirmed the superior quality of larvae displaying pronounced phototactism, expressed in better survival rates, but also highlighted that, even for newly hatched larvae with a good initial condition, subsequent survival and growth depend on conditions of optimal nutrition. Swimming capacity has also been used to evaluate larval quality (Treece & Fox, 1993). However, it seems obvious that poor swimming ability expresses an inferior physiological condition, illness or any other type of stress induced in larvae, which makes them unsuitable for larval culture.

6.4.4 Productive criteria

Survival through larval development is regularly monitored during larval culture trials, with lower values being interpreted as indicators of poor larval quality (Bray & Lawrence, 1992). Based on the high correlation between survival to postlarvae and early larval survival, Hernández-Herrera *et al.* (2001) have proposed for penaeid shrimp the establishment of a 'cut-off point' beyond which culture of a specific larval batch should be discontinued. This approach may be of particular interest for marine ornamental shrimps, since this would avoid wasting time and money culturing larvae evidencing inadequate survival rates. Setting a 'cut-off point' for larvae under culture is even more relevant if we consider the ability of caridean and stenopodidean larvae to delay development and metamorphosis (e.g. mark-time and terminal additive moulting). This delay in larval development extends culture periods for several extra weeks and commonly results in the production of only a reduced number of poor quality juveniles (Calado *et al.*, 2005a).

6.4.5 Survival in stress tests

Stress tests are commonly employed to evaluate the quality of early juvenile shrimp rather than their previous larval stages, with the salinity stress test

being by far the most commonly employed (Racotta *et al*., 2003; Palacios & Racotta, 2007). However, given the marine origin of ornamental shrimps, in opposition to the estuarine nature of most cultured penaeids, salinity stress tests may not be a reliable option to evaluate either their larval or juvenile quality. The suitability of larval exposure to high ammonia concentrations [e.g. 20 mg/litre of total ammonia for 24 hours (Racotta *et al*., 2004)] as a stress test has been evaluated to determine if it could be a good indicator of larval quality from batches produced by broodstock in captivity (Cavalli *et al*., 1999, 2000a). Recorded results indicate that these tests are sensitive and reproducible, providing evidence that zoeal survival to ammonia stress tests is related to survival of early juveniles during grow-out (Cavalli *et al*., 2000b; Hernández-Herrera *et al*., 2001; Racotta *et al*., 2004). In this way, ammonia stress tests appear to be a feasible criterion for evaluating larval quality that may be readily employed in the culture of marine ornamental shrimps.

Chapter 7

Larval Culture

7.1 Larval culture systems

Culturing marine invertebrate larvae has always been considered a challenging task, requiring the construction and operation of more or less complex systems. Decapod crustaceans are certainly no exception to the rule and their culture has deserved the attention of several researchers in recent decades. The existence of significant differences in larval morphology among different groups of decapods has become an extra problem in the conception of suitable larval culture systems. Apart from the general requirements already highlighted for life support systems designed for keeping reproductive shrimps in captivity, these systems must address the specific needs displayed by decapod larvae under culture.

Despite the earlier belief expressed by researchers on how marine ornamental shrimp larvae could be cultured using standard techniques developed for the penaeid shrimp industry (Fletcher *et al.*, 1995), later works have clearly demonstrated the need to develop specific culture systems and methodologies. Spiny lobster and marine ornamental shrimp larval culture display several common requirements, which have allowed researchers to adopt and/or adapt existing culture systems. Just like marine ornamental shrimps, spiny lobster larvae (the phyllosoma) also display frail larval appendages, long larval cycles, the ability to delay larval development under unsuitable culture conditions and a susceptibility to microbiological infections when exposed to prolonged culture periods (Calado *et al.*, 2003a). Larval culture of marine ornamental shrimps may be achieved in still or circulating water. Culture in still water is commonly employed when manipulating a reduced number of larvae (usually less than 100) and employing small water volumes (usually between 20 and 20 000 ml). This culture technique is commonly used when evaluating specific larval requirements and/or traits, such as larval stage duration, morphological changes through larval development, resistance to starvation and feeding rates (Zhang *et al.*, 1997b, 1998b, c; Calado *et al.*, 2001a; Simões *et al.*, 2002). However,

still water culture systems are unsuitable for culturing decapods when scaled up, even when performing daily water changes to ensure water quality. The main problem is the passive sinking of cultured larvae, which, among other negative effects, increases the chances of 'tangle' damage in larval appendages and of microbiological infections, mainly due to the aggregation of larvae, food and debris in the tank bottom (Kittaka, 2000). Water quality deterioration and high chemical oxygen demand are also important drawbacks resulting from the use of still water systems (Kittaka, 1994a). Larval culture systems operating with circulating water may be run on a flow-through or recirculated basis. Flow-through culture systems may significantly decrease the risks of larval exposure to poor water quality commonly induced by the accumulation of nitrogenous wastes from larval metabolism and decaying uneaten food (Ritar, 2001). However, such systems may only be operated in near-shore facilities using sea water with reliable quality during the whole year. Unfortunately, it is not uncommon to experience seasonal blooms of unwanted phyto- and zooplankton when operating flow-through systems, even when employing suitable filtration (e.g. canister filters, ozonation and UV irradiation) to treat newly pumped sea water. Certain zooplankton blooms, such as the free-swimming life stage of hydroids, may have devastating effects in larval culture systems. These organisms are voracious predators, capturing large numbers of cultured larvae (and larval prey) in only a few hours, heavily infesting culture systems and seriously threatening profitability. The eradication of these organisms from larval culture systems is virtually impossible, only being achieved after stopping and disinfecting the system. Filling the system with fresh water, and consequently destroying any existing beneficial microbiological communities, is a drastic solution that completely eradicates these pest organisms (Calado, unpubl. obs). Flow-through systems may also be more vulnerable to pollutants (e.g. heavy metals, pesticides) diluted in nearby marine waters, which may go unnoticed until they enter larval culture systems and induce mass mortality.

Recirculated larval culture systems are the common choice for marine ornamental shrimps since they can easily be set up in small inland areas and operated with either natural or synthetic sea water. The use of natural sea water is sometimes advocated over the use of synthetic water. None the less, natural sea water continues to present the same risks described above for flow-through systems. However, water quality, as well as the presence of unwanted pest organisms, can be more easily controlled in recirculated systems, since 'new' water will only be deliberately introduced in the system when performing partial water changes. In the culture of decapod crustaceans the successful replacement of natural by synthetic sea water is directly affected by the quality of the selected salt mix. When a suitable salt mix is employed, culture results may even outperform those recorded using natural sea water (Gallagher & Brown, 1976). Additionally, when using synthetic sea water prepared with a high-grade salt mix and fresh water purified by a reverse osmosis unit, the

risk of involuntarily introducing pathogenic agents to larval culture systems is significantly reduced.

Tank shape and water circulation dynamics are two of the most relevant features of successful culture systems for larval decapods. For this reason these two features need to be properly checked when testing the suitability of new larval culture tanks. Additional aspects that must also be considered include tank color and functionality, namely routine tank cleaning, periodic larval transfer and the supply and replacement of dietary live prey. The building and operational cost of each larval culture tank is also an important issue and needs to be conscientiously analysed when designing these units. Intricate larval culture tanks are commonly too expensive to scale up, which prevents their commercial use (Ritar, 2001). Sometimes, prototype units can be so prohibitively expensive that not even small laboratory-scale experimentation can be addressed because the required financial investment prevents proper replication.

The most 'inspiring' larval culture tank design for the frail larval stages of decapod crustaceans was the 'planktonkreisel'. The 'planktonkreisel' was first described by Greve (1968), and was originally destined for ecological studies on ctenophores. Its innovative design was intended to overcome the difficulties commonly faced when trying to culture planktonic organisms:

(1) the loss of their locomotory activity;
(2) the physical damage induced to these organisms when kept in systems with inadequate water dynamics, promoting frequent 'high-speed' contact with the tank walls and bottom;
(3) the damage induced by air bubbles when employing this method to provide water motion;
(4) the suction damage induced by screens placed in the tank outflow; and
(5) the recurrent use of unsuitable rectangular-shaped aquaria to stock these organisms, commonly resulting in high density aggregations in reduced areas, promoting 'tangle' damage and agonistic interactions.

The round shape of the planktonkreisel combined with its central column [inspired by the 'inside filter arrangement' of Flüchter (1964)] provided suitable water filtration as did the continuous rotation of the water in the culture medium.

Despite its original purpose, Greve (1968) highlighted the potential displayed by the planktonkreisel for the captive keeping and culture of several other planktonic organisms, including crustaceans.

Larval culture systems based on the 'planktonkreisel' have been successfully employed to culture clawed lobsters (Hughes *et al*., 1974) and spiny lobsters (Kittaka, 1994a), keeping both larvae and food in suspension through the upwelling motion of the water. However, when cultured organisms display

long larval development periods, such as spiny lobsters and marine ornamental shrimps in the genera *Stenopus* and *Lysmata*, these systems are considered inadequate because proper tank cleaning cannot be performed. This drawback was early pointed out by Phillips & Sastry (1980), and regular tank cleaning became one of the main concerns in the design of upwelling culture tanks. The culture tanks for spiny lobster larvae designed by Illingworth *et al.* (1997) were fitted together in a block of four units connected through transfer ports. These ports allowed larvae to be moved from one tank to another simply by opening them and adjusting the water flow. Three tanks could be in operation simultaneously, with the fourth tank being kept empty to allow the reception of larvae from the culturing unit that needed to be cleaned. This procedure of larval transfer allowed culture tanks to be regularly cleaned, and eliminated the tedious task of manually handling cultured larvae. Additionally, the outflow of these larval culture tanks was equipped with screens displaying variable mesh sizes specially designed to optimize feeding protocols. Screens with smaller mesh sizes (also known as feeding screens) were employed to prevent larval prey from being flushed from the tank. Daily, these screens were replaced by similar ones displaying larger mesh sizes (cleaning screens), allowing uneaten larval prey to be flushed. When only a reduced number of 'older' larval prey could be detected in the larval culture tank, cleaning screens were replaced by feeding screens and new dietary prey could be supplied to cultured larvae [see Illingworth *et al.* (1997) for a detailed description of the system design and operation]. Despite the potential exhibited by these rearing tanks, their intricate design and high financial cost led researchers to seek alternative solutions that would allow them to perform replicated comparisons of experimental culture trials (Ritar, 2001). Since then, several tank designs for spiny lobster larval culture have been employed with variable degrees of success: circular tanks with flat bottoms (Ritar, 2001), planktonkreisel-like tanks (Kittaka, 2000), hemispherical tanks (Sekine *et al.*, 2000) and elliptical tanks with concave bottoms (Matsuda & Takenouchi, 2005).

The preliminary observations of Fletcher *et al.* (1995) on the culture of three highly popular marine ornamental shrimps (*Stenopus hispidus*, *Lysmata amboinensis* and *L. debelius*) do not specify which type of larval culture tanks were employed.

Palmtag & Holt (2001) proposed a larval culture system for marine ornamental shrimps employing 400-liter round tanks equipped with external biofilters. In each 400-liter tank were immersed 18-liter cylindrical rearing chambers, with the openings on the sides and bottom of the chambers being covered with mesh cloth. These lateral and bottom openings allowed water flow and retained larvae and dietary prey inside the rearing chamber. An interesting feature of this culture system was the use of feeding rings with strips of shrimp tissue suspended in the rearing chambers; this dietary item apparently played a key role in the success of culture trials. This system has allowed researchers to perform highly successful trials on marine ornamental shrimp

larval culture, establishing the first descriptive and repeatable protocols to raise the highly prized fire shrimp *L. debelius* to the juvenile stage.

The first recirculated larval culture system that gained popularity for the culture of marine ornamental decapods was that of Calado *et al.* (2003a) employing cylindrico-conical tanks. This system was also inspired by Greve's (1968) planktonkreisel, as well as by the upwelling spiny lobster larval culture tanks described by Illingworth *et al.* (1997). Calado *et al.*'s (2003a) system also employs feeding and cleaning screens (as previously described for the culture system of Illingworth *et al.*, 1997). Palmtag & Holt (2007) highlighted how this larval culture system could further improve the results already achieved by these authors by employing mesh rearing chambers immersed in 400-liter round tanks. Calado *et al.* (2003a) point out how enabling uneaten larval prey to be flushed daily and replaced by new ones exhibiting a higher nutritional profile may play a crucial role in the success of culture trials employing this rearing system. In this recirculated system, water is gravity fed to each culture tank, the inlet being connected to a valve regulating water flow and positioned in the deepest point of the rearing tank. Small research-scale (12-liter) (see color plate 7) and large commercial-scale (200-liter) larval culture tanks were successfully employed to culture several marine ornamental decapod species, with emphasis on the Monaco shrimp *Lysmata seticaudata*. Although the larval culture of this *Lysmata* species seems to be less demanding than that of other related species (e.g. *L. amboinensis* and *L. debelius*), previous studies using different culture systems have never been able to successfully produce a single juvenile shrimp (e.g. Couturier-Bhaud, 1974). Small research-scale tanks are suitable for laboratory trials requiring a large number of units for robust replication and testing of experimental conditions. On the other hand, larger tanks seem to be the best option for enterprises willing to culture marine ornamental shrimps for commercial purposes.

An interesting feature of commercial-scale culture tanks was the use of several artificial grass strips suspended in the water column, which not only increased the tank settlement area but also provided suitable shelter from light for the negatively phototactic megalopa and juvenile shrimps. These strips were also used to easily remove newly settled shrimps from the culture tank, since they firmly cling to these structures. This simple procedure avoided the need to drain the culture tank (through the bottom valve placed in the tank's deepest point), an operation that commonly damages the frail larval appendages of late zoeal stages which may still be present in the culture vessel. Artificial grass strips also increase the area available for the development of beneficial biofilms, a feature known to improve the performance of culture systems for marine shrimps (Arnold *et al.*, 2006; Ballester *et al.*, 2007).

Although large culture tanks were initially designed to address the needs of enterprises targeting commercial-scale culture of marine ornamental shrimps, their acceptance for the industry has not been totally satisfactory. One of the drawbacks of these large culture units is the need for a considerable number of

larvae to stock these tanks appropriately and optimize their use. Since caridean and stenopodidean shrimps produce considerably smaller larval batches than penaeid shrimps or lobsters, smaller tank volumes may be preferred over 200-liter units. If large volume tanks are used, a significantly higher number of reproductive pairs must be stocked, since tank use optimization will only be achieved if several larval batches are pooled. None the less, this approach will only be suitable if larval batches hatch with no more than a 3-day difference between them, in order to prevent potential cannibalism in later larval stages and/or asynchronous settlement. Another problem commonly associated with large-scale culture tanks for marine ornamental shrimp larvae is routine cleaning. Siphoning dead larvae and any larger particles of uneaten food can be time-consuming and the use of daily purges using the bottom valve of culture tanks always drains and damages healthy larvae. Contamination by pest organisms, such as hydroids, is also a serious problem when employing large-scale culture tanks since it is virtually impossible to control outbreaks. An outbreak of hydroids in large culture systems always results in the predation of thousands of larvae under culture in less than 3 or 4 days, seriously affecting the commercial viability of marine ornamental shrimp culture. This particular aspect is probably the main reason why enterprises commonly choose smaller volume culture tanks (10 to 50 liters) over larger ones. In order to decrease the risks described above when using large larval culture tanks, enterprises are willing to accept the extra work associated with routine tasks required for the proper keeping of several smaller tanks.

Despite the popularity achieved by the tanks devised by Calado *et al.* (2003a) for marine ornamental shrimp larval culture research, there is still room for more improvements in order to reduce significantly the time required for carrying out routine tasks and increasing functionality for the culture of later zoeal stages. The method described in the system of Calado *et al.* (2003a) for changing cleaning and feeding mesh screens can be simplified, eliminating the need to displace water to lower its level below the outlet. Attaching a cleaning and a feeding mesh screen at the opposite ends of a PVC 'T' and then fitting the free portion of the 'T' to the outlet allows both screens to be replaced simply by rotating the 'T' and the screens (Figure 7.1). If the screens are simply fitted, and not glued, to the 'T', the screen not being employed (remaining outside the water) can be detached and properly cleaned to avoid clogging.

The feeding rings described by Palmtag & Holt (2007) can also be easily adapted to the Calado *et al.* (2003a) system.

The cylindrico-conical shape of culture tanks may not be the most suitable for culturing the late zoeal stages of ornamental shrimps. These larger, and consequently heavier, larvae are commonly seen 'sliding' towards the deepest point of culture tanks along their conical slopes and being 'struck' by upwelling water entering the tank. This mechanical stress is known to be responsible for damaging larval appendages, namely the large paddle-shaped fifth pereiopods of late *Lysmata* larvae and the long rostrums of *Stenopus*

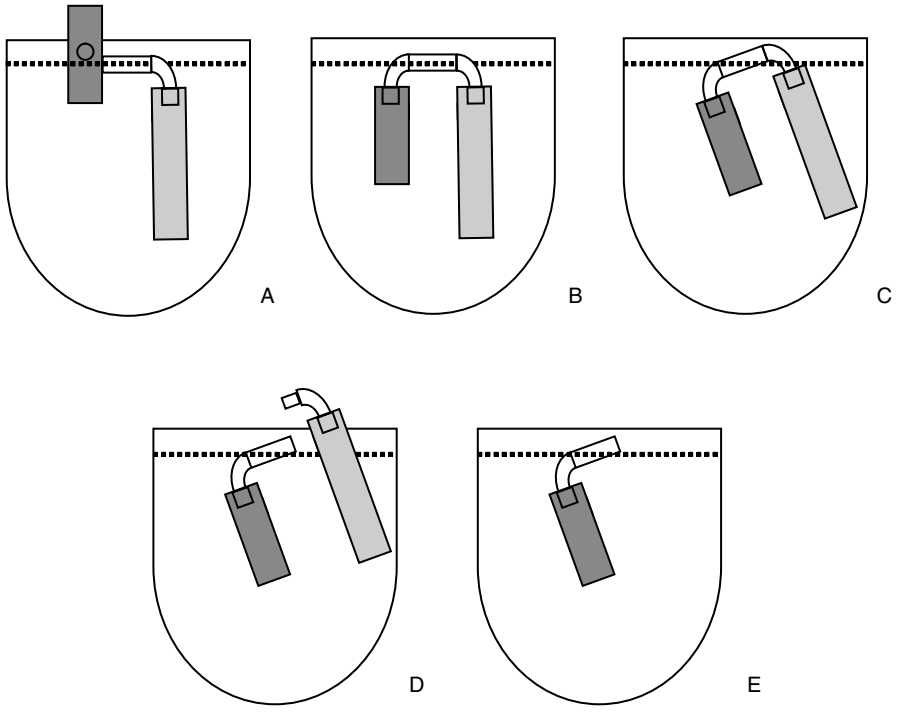


Figure 7.1 Screen changing procedure. (A) The cylindrico-spherical rearing tank has a large mesh screen (500 μm) (dark grey) and a fine mesh screen (150 μm) (light grey) placed simultaneously with the larger mesh screen being only partly submerged and with its outlet 15 mm above the water surface so that larval prey are not flushed; (B) the larger mesh screen is totally submerged; (C) the outlet PVC 'T' connecting both screens is partially twisted, so that the end connecting to the fine mesh screen remains above the water level; (D) the thinner mesh screen is detached from the outlet PVC 'T', allowing it to be cleaned whenever required; (E) uneaten larval prey is allowed to flush through the larger mesh screen.

larvae. Preliminary trials using cylindrico-spherical tanks (Figure 7.2) have revealed that this mechanical stress is significantly reduced when using this particular tank shape since upwelling water is more evenly dispersed. The elliptical tanks with concave bottoms used by Matsuda & Takenouchi (2005) to culture late-stage spiny lobsters may also display good performance if employed to culture marine ornamental shrimps.

The impact of tank coloration on culture of decapod crustacean larvae remains controversial, although recent work has shown that background color can significantly affect larval survival and development of decapod crustacean species (Rabbani & Zeng, 2005). So far, black, as well as white, tanks have already been successfully used to raise marine ornamental shrimp larvae. However, no study has ever addressed the relevance of background coloration in the larval culture of these species. Yasharian *et al.* (2005) reported how some *Macrobrachium rosenbergii* producers claim that dark (black, blue or green) interior tank walls promote higher larval survival. The main

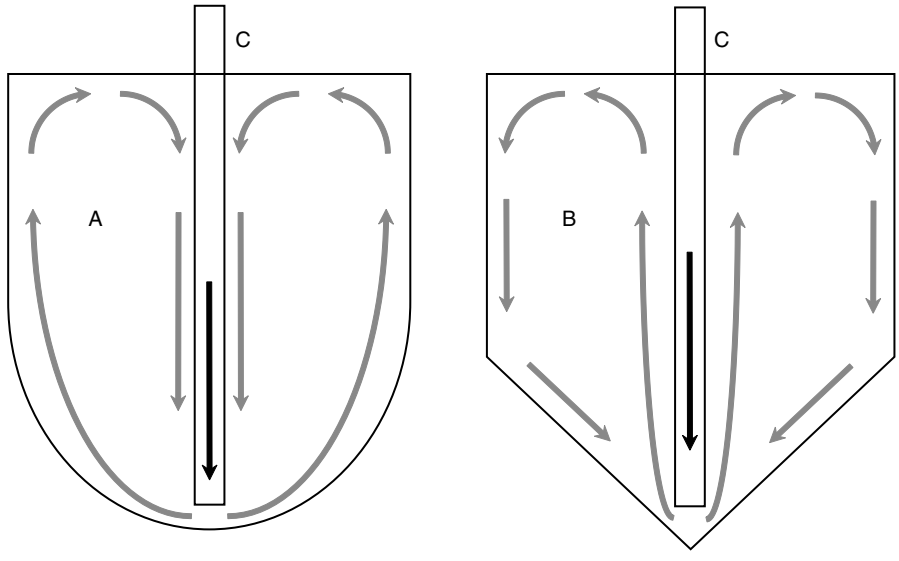


Figure 7.2 Larval rearing tanks: A, cylindrico-spherical tank; B, cylindrico-conical tank; C, inflow pipe; black arrows represent inflowing water; grey arrows represent predominant water flow inside the rearing tank.

reasons given to support this claim are that cultured larvae may detect dietary prey more easily against a dark background and that larval and prey distribution is more uniform, thus ensuring a higher number of feeding encounters. However, other producers claim that larvae are not visual predators, and instead find their prey through tactile responses rather than sight, and that white tanks present the advantage of making routine larval surveys easier as well as facilitating the monitoring of tank cleanliness (see Rodrigues *et al*., 1998). Rabbani & Zeng (2005) evaluated the effect of five different colors (black, dark green, maroon, sky blue and white) on larval survival and development of the crab *Scylla serrata* (Forskål, 1775), and found that darker colored backgrounds promoted higher survival. The authors also verified that larvae cultured in darker backgrounds commonly displayed a shorter and more synchronous larval development. The main reason given by the authors to justify the differences recorded was an increase in the feeding efficiency of larvae cultured in darker tanks. Lin & Omori (1993) remarked that background colors may be species specific, with certain colors possibly representing chronic stressors for cultured larvae. In their study, the speed and distance of horizontal movement of *M. rosenbergii* larvae stocked in white tanks increased three-fold in comparison with those placed in darker containers. Lin & Omori (1993) suggested that excess 'excitation' in lighter colored containers may be responsible for decreased feeding rates and consequently lower larval survival, mainly due to an increase in energy consumption.

The apparently inconclusive results recorded on the suitability of background color for decapod crustacean larval culture may be related to hypothetical shifts in phototactic responses and feeding strategies during larval development. As an example, different larval stages of spiny lobster larvae display different phototactic responses (Rimmer & Phillips, 1979), with earlier stages exhibiting positive responses (Ritz, 1972) and later stages being negatively phototactic (Phillips & Sastry, 1980). As cultured larvae advance to later stages, their visual acuity and active predatory behavior also increase, emphasizing the importance of adequate background coloration to enhance larval ability to contrast food items (Yasharian *et al*., 2005). Future studies should be aimed at clarifying the relevance of background color in marine ornamental shrimp larval culture during early and late zoeal stages, since optimizing this aspect may allow enterprises to increase survival to metamorphosis, decrease production costs and increase profitability.

7.2 Culture procedures and selection of high quality larvae

The success of decapod larval culture is assumed to be directly influenced by larval and water quality (namely the absence of nitrogenous wastes and pathogenic agents) and by the existence of suitable larval food (both in quantity and quality), as well as by the ability of cultured larvae to successfully capture and ingest dietary items.

7.2.1 Larval quality and culture tank management

Although larval quality plays a crucial role in the success of any larval culture trial, determining the quality of newly hatched larval batches is far from being an easy task. As mentioned in the previous chapter, shrimp larval quality is highly dependent on broodstock condition (Racotta *et al*., 2003). Poor maturation diets and husbandry conditions for reproductive pairs invariably result in poor quality larvae, which already display high mortality rates in earlier zoeal stages. The most widely used methods to evaluate larval quality of aquatic organisms usually involve exposing them to short but extreme environmental stress. Salinity, temperature, pH, formalin or ammonia stress tests are commonly employed to evaluate larval shrimp quality (Cavalli *et al*., 2000b). In these situations, larval physiological condition determines larval ability to survive (Briggs, 1992; Dhert *et al*., 1992; Samocha *et al*., 1998). However, most of these tests were developed to evaluate the quality of late-stage larvae or early-stage juveniles as they are unsatisfactory for estimating the quality of shrimp larvae immediately after hatching. From the various tests available to ascertain larval quality (behavioral, physiological and biochemical), light response and swimming ability are the most widely used in

marine ornamental shrimp larval culture (e.g. Simões *et al*., 2002; Calado *et al*., 2003a, 2005a, 2007c). Browdy (1992) also considered phototactic responses as a suitable criterion to evaluate the quality of penaeid nauplii. Singh & Philip (1995) followed a similar approach to separate 'healthy' from 'weak' larvae of *M. rosenbergii*. Only newly hatched larvae showing a positive response towards a light source and simultaneously displaying strong swimming ability are generally selected for culture. These criteria have gained popularity among researchers as well as commercial producers, probably because they can be more readily and simply employed than biochemical or stress tests. None the less, phototactic responses are usually considered to be an indirect indicator of larval quality. Ibarra *et al.* (1998) verified that, irrespective of positive phototactic responses, larval survival and growth depend on optimal nutrition and culture conditions. Although less commonly employed, morphological criteria may also provide feasible data on larval quality. Batches of newly hatched larvae showing a pale coloration or an 'abnormal' looking body shape usually exhibit poor performance during culture trials. Typical morphological signs of poor quality larvae are bent abdomens, a curved (rather than straight) rostrum and 'uninflated' carapace spines. When larval batches showing such morphological signs are selected for culture trials, larval mortality may reach 90 to 100% in only 48 hours, even when high quality dietary prey are provided (Calado, unpubl. obs.).

However, even when starting culture trials with high quality larvae, final survival results may be far from being optimal. The most common causes of poor performance are problems related to water quality and the suitability of dietary items. Several researchers have erroneously advocated that marine ornamental shrimp larvae do not need to be fed immediately after hatching, since they possess enough energetic reserves to advance to the next zoeal stage in the absence of food (Fletcher *et al*., 1995; Zhang *et al*., 1998b). Although decapod larvae in the wild are exposed to variable periods of starvation, mainly due to patchiness of the food supply, prolonged starvation periods always promote deleterious effects (Paschke *et al*., 2004; Gimenez & Anger, 2005). Calado *et al.* (2007c) have shown how different species in the genus *Lysmata* may display different tolerance levels to variable starvation periods immediately after hatching. The authors have verified that *L. amboinensis* and *L. debelius* are more negatively affected by initial starvation periods than other *Lysmata* species displaying facultative primary lecithotrophy. However, even species displaying facultative primary lecithotrophy may exhibit poorer culture performances if exposed to total starvation during the first zoeal stage (Calado *et al*., 2005a). Therefore, it is always vital to provide adequate food to newly hatched larvae, in order to ensure that larval quality is not negatively affected by unsuitable husbandry practices.

Marine ornamental shrimp larvae are able to delay larval development for long periods of time (usually several weeks) when placed in unsuitable culture conditions (Fletcher *et al*., 1995; Calado *et al*., 2001a). For this reason,

during larval culture trials it may be necessary to determine when culture tanks should be discontinued. Empirical data seem to indicate that if a mortality of at least 50% of cultured larvae has already occurred when reaching half of their larval development, surviving larvae will also experience heavy mortalities in the next zoeal stages, and late zoeal stages (commonly <5% of the initial stocked number of larvae) will display mark-time or terminal additive moulting. As a practical example: if a larval batch of *L. seticaudata*, which undergoes nine zoeal stages before metamorphosis, has already experienced a mortality of at least 50% when reaching the fifth zoeal stage, the larval culture tank should be discontinued. In some commercial enterprises, it is a common practice to pool in a single culture tank late-stage surviving larvae, which are delaying metamorphosis, in an attempt to minimize the financial costs associated with the culture of poor larval batches. This option is particularly common when culturing shrimp species of higher market value, namely *L. debelius*.

During culture trials, a regular survey of larval stages and their respective duration may help to diagnose the existence of unsuitable rearing conditions. Suboptimal culture conditions promote the occurrence of prolonged stage duration and the appearance of extra instars (e.g. mark-time moulting and terminally additive stages). Calado *et al.* (2001a) verified that *L. seticaudata* larvae fed a suboptimal diet (decapsulated *Artemia* cysts) did not die, but could not develop beyond the fifth zoeal stage. Larvae being fed this inadequate diet performed over six consecutive moults, in which no significant morphological differences could be recorded (compared with a 'typical' zoea V). However, if larval diet is optimized, or any other stressful rearing condition has been corrected, larvae displaying mark-time moulting and/or terminally additive stages may reassume their regular larval development pattern and successfully metamorphose. Wunsch (1996) recorded that *L. amboinensis* larvae displayed variable durations for different zoeal stages, with considerably longer intermoult periods being recorded for later zoeal stages. This pattern is also a good indicator of inadequate culture conditions, namely nutritional issues, although it must also be highlighted that variable culture temperatures significantly influence larval stage duration (Anger, 2001). At optimal and stable culture temperatures, marine ornamental shrimp larvae display shorter larval stage duration, but never less than 2 days per stage (Calado *et al.*, 2005a). In this way, it is possible to calculate the minimum larval culture period that can be expected for a particular species, as long as the total number of zoeal stages is known. Unfortunately, complete and standardized morphological descriptions of the different zoeal stages occurring during larval development of the most popular marine ornamental shrimps are still largely missing (Calado *et al.*, 2003b). As an example, larvae of a marine ornamental shrimp being cultured in optimal conditions, displaying a total of nine zoeal stages during its larval development, will be expected to settle 19 days after hatching (9 zoeae $\times$ 2 days duration per zoeal stage = 18 days of zoeal development = settlement occurring at the 19th day post-hatching).

Swimming behavior of cultured larvae can also provide valuable information concerning larval health, and help identify unsuitable rearing conditions. Larvae 'drifting' with water inflow, or permanently remaining near the bottom of culture tanks without expressing active swimming behavior are usually experiencing suboptimal conditions. Subsequent analysis of these larvae commonly reveals heavy loads of microorganisms fouling the body and larval appendages, giving them a 'milky' appearance. *Lysmata* larvae display a typical looping behavior when feeding in culture tanks, holding their fifth pereiopods completely straight (or slightly bent) and performing a complete backward circle with the tip of the dactyls of pereipod 5 as the central point (Rufino & Jones, 2001b). Since even larvae missing the fifth pair of pereiopods display this peculiar swimming behavior, the absence of looping by *Lysmata* larvae in culture tanks can be regarded as a warning sign of suboptimal conditions.

Another practical approach employed in commercial facilities is to periodically (daily or every other day) remove newly settled shrimps, increasing the number of larval prey available for late-stage larvae which have still not metamorphosed. This procedure also decreases the risk of newly settled shrimps cannibalizing late-stage larvae. Another drawback of using poor quality larval batches, or exposing good quality larvae to unsuitable culture conditions, is the occurrence of an asynchronous settlement pattern (Calado *et al*., 2005a). Fletcher *et al.* (1995) report an extreme situation of asynchronous settlement for *Stenopus hispidus*, with larvae from the same batch being more than 12 weeks apart in settlement time. Early and late metamorphosed post-larvae of ornamental shrimps display significant differences in total length (Calado *et al*., 2005a). Castille *et al.* (1993) proposed that a coefficient of size variation displayed by newly settled post-larvae could be a good indicator of their quality. However, Racotta *et al.* (2004) note that post-larval length is not a good indicator because many other variables can affect post-larval quality.

For species for which there is already life-history information available from the field, it may be possible to indirectly evaluate the suitability of the culture conditions employed. Using *S. hispidus* as an example, it is known that this species is able to delay metamorphosis in the presence of unsuitable conditions, either in the wild (Gurney & Lebour, 1941; Williamson, 1976) or in culture trials in captivity (Fletcher *et al*., 1995). In both scenarios, late-stage larvae delaying metamorphosis do not undergo significant morphological changes but continue to increase in body size, eventually becoming 'giant larvae' (as defined by Gurney, 1942). Larval size obviously influences the size of the newly settled megalopa. Chockley & St. Mary (2003) reported that *S. hispidus* in the upper Florida Keys settle at a consistent total length of approximately 17 mm. If the size at settlement recorded in culture trials of *S. hispidus* is larger than that recorded in the wild, it may indicate that cultured larvae were larger than wild ones and therefore were probably exposed to suboptimal conditions. Nevertheless, it must be stressed that this is just a potential criterion to

evaluate the suitability of culture conditions and that more or less significant variations may occur in the settlement size of the same species in different geographical regions (Chockley & St. Mary, 2003).

The effect of asynchronous settlement patterns in post-larval quality is still unknown for ornamental shrimps. However, the differences recorded in post-larval size certainly pose a challenge for successful grow-out. Ahvenharju & Ruohonen (2007) suggest that social environments in which there is a wide variation in carapace length between individuals promote a higher number of agonistic behaviors. These interactions between different sized individuals may promote higher mortality during grow-out.

Regular tank cleaning is essential to ensure good water quality for cultured larvae, particularly in recirculated systems. Additionally, it is an effective way to minimize the proliferation of microbiological communities that may negatively affect cultured organisms. When employing larval culture tanks equipped with replaceable screens allowing uneaten live prey to be flushed daily from culture tanks, routine cleaning must address the removal of dead larvae, old exuvia and larger pieces of uneaten food that cannot be drained through the mesh screens. Usually, a simple piece of air hose is used to siphon off any of the items detailed above. When culturing early zoeal stages, a small mesh screen is usually fastened to the extreme end of the hose placed inside the tank to prevent larvae from being siphoned. Siphoning larvae, even through large-diameter hoses, commonly induces some sort of damage to their frail appendages, particularly in late zoeal stages. It is therefore advisable to use small beakers to transport live larvae between culture tanks and collect only a small number of specimens (five to ten) to prevent the occurrence of 'tangling' damage.

7.2.2 Water quality

Water quality issues concerning the presence of high concentrations of nitrogenous wastes in recirculated marine systems have already been highlighted in previous sections. None the less, it is important to stress that during larval development the negative influence of inadequate levels of nitrogenous compounds is even more pronounced, owing to their interference in the complex metabolic pathways regulating morphogenesis, growth and moult cycles of developing larvae. Apart from implementing suitable biological filtration systems, the most common procedure to ensure good water quality in recirculated systems is the performance of periodic partial water changes. This approach relies on the principle of 'solution to pollution is dilution', although it is only effective as long as it does not promote any abrupt changes in other key water parameters such as temperature, salinity and pH.

Calcium concentration and pH values should be closely monitored during larval culture because of the periodic moults that larvae need to undergo from hatching until settlement. During the premoult period, Ca^{2+} is reabsorbed

from the old cuticle, which is going to be discarded. The new cuticle is primarily mineralized with external (environmental) Ca^{2+}, as well as internal stores, with different specialized crustacean epithelia being responsible for the bidirectional exchange of Ca^{2+} (Wheatly, 1999). Carapace mineralization can be described as the formation of calcium carbonate and hydrogen ions from calcium and bicarbonate ions in the cuticle (Perry *et al*., 2001). Although the reaction is reversible, high pH values (e.g. pH = 8.2) favour the deposition of calcium carbonate (Cameron, 1985). In this way, the calcification process is sensitive to pH differences, with low pH levels in sea water retarding the diffusion of H^+ ions from the blood, eventually inducing acidosis and in extreme cases even death. In recirculated systems with considerable numbers of developing crustacean larvae, calcium levels may be rapidly depleted, while pH values may also experience a sharp decrease due to the accumulation of several acidic compounds resulting from the metabolism of larvae and dietary prey. These two factors may disrupt calcium homeostasis in cultured larvae and promote mortality during ecdysis. A typical warning signal concerning calcium and/or pH imbalances is the incapacity of moulting larvae to fully discard their old exoskeleton. These larvae are unable to completely remove their pereiopods and antennae from the exuvia, exhibit erratic swimming patterns and commonly die before the next moult cycle.

7.2.3 Microbiological aspects

For routine sanitation, larval rearing tanks should be cleaned, disinfected and dried between culture trials. With small tanks, such as the ones commonly employed for ornamental shrimp culture, it is advisable that after finishing a culture trial the following procedure is applied (OIE, 2003):

(1) fill the tank with fresh water to its maximum capacity;
(2) immerse all nonporous and water-resistant equipment in the tank and add calcium hypochlorite until a minimum of 200 ppm of free chlorine concentration is reached;
(3) let the tank settle overnight;
(4) drain the tank and rinse it with fresh water (please note that for environmental reasons drained water should be dechlorinated before being disposed of);
(5) allow the tank to dry completely; and
(6) fill the tank and flush it to ensure that all chlorine residues are eliminated before restocking the tank for another trial.

The occurrence of sudden high mortalities induced by contamination by microorganisms during larval culture of decapod species that display long larval cycles has been well documented, particularly for spiny lobsters

(Bourne *et al*., 2004; Ritar *et al*., 2006). These contaminations may occur through the use of unsuitable sea water (either natural or synthetic), and through the introduction of newly hatched larvae and larval prey that have not been previously disinfected (e.g. Austin & Allen, 1982). Fouling by epibiont organisms of decapod larvae negatively affects larval swimming and feeding ability, directly or indirectly promoting mortality (McAllen & Scott, 2000). Ritar *et al.* (2006) described how epibionts infesting spiny lobster larvae can increase larval body density and how a decrease in larval swimming efficiency results in higher chances of contact with surface biofilms present in culture tanks, eventually promoting further bacterial attachment in swimming appendages. Epibionts can also extend larval inter-moult phase beyond tolerable levels, commonly resulting in the death of the larva during ecdysis. Obviously, this is a highly relevant aspect for the larval culture of decapod species already displaying long larval development periods. The enteric disease state, which is often recorded in late-stage larvae failing to reach metamorphosis, is associated with the presence of significant numbers of *Vibrio* spp., with *Artemia* nauplii and metanauplii being considered as potential sources of fouling organisms (Handlinger *et al*., 1999).

In this way the development of culture protocols for marine ornamental shrimp larvae may only be properly addressed if suitable techniques to reduce bacterial contamination of newly hatched and cultured larvae are also developed.

Ozone is known to be a powerful oxidizing agent, and is used to eliminate viral, bacterial and fungal pathogens (Summerfelt & Hochheimer, 1997; Suantika *et al*., 2001). Additionally, ozonation has already been shown to be highly effective in the aquaculture of crustaceans (Danald *et al*., 1979; Thiesen *et al*., 1998), being routinely used for the disinfection of eggs (Coman *et al*., 2005; Sellars *et al*., 2005). However, unlike penaeids, caridean and stenopodidean shrimps incubate their eggs in the abdomen during the entire embryonic development period, which impairs the use of this disinfection technique. It is known that the most important reaction in sea water during ozonation is the initial oxidation of bromide ions (Br^-) to hypobromite ions (OBr^-), which may be further oxidized to form bromate ions (BrO_3^-) (Buchan *et al*., 2005). Tango & Gagnon (2003) pointed out that, in marine water, bromide will not react with ozone to form the toxic bromate as long as ammonia is present. However, it is well known that ammonia levels should be kept as close to zero as possible in recirculated systems, thus favouring the excessive production of bromate. Since this compound is relatively stable in marine water (Richardson *et al*., 1981), it may accumulate in recirculated systems. Even when employing synthetic sea water, in which bromide ions may be absent, the accumulation of this toxic compound is still recorded in recirculated systems (Grguric *et al*., 1994). Bromate is classified as carcinogenic to human health, is known to be a potent toxin for aquatic life, and most probably is also toxic to crustacean larvae. The beneficial effects of water ozonation during

decapod larval culture, as well as larval vulnerability to inadequate levels of ozonation, were well demonstrated by Ritar *et al.* (2006) for larval spiny lobsters. Phyllosomas reared in sea water ozonated at low and moderate levels [400 and 500 mV oxidation–reduction potential (ORP)] displayed higher survival and lower bacterial contamination than larvae cultured in unozonated water. At high ozonation (600 mV), all larvae suffered deformities, starved and ended up dying. The same authors also recorded how filtering ozonated water through activated charcoal and coral sand significantly increased larval survival. However, later larval stages seemed less tolerant of high ORP values and had to be cultured in unozonated water until metamorphosis. Water ozonation may also play an important role in the culture of marine ornamental shrimps displaying long larval development, since it can minimize mortality induced by body fouling or pathogenic agents. However, as pointed out by Ritar *et al.* (2006), keeping larval crustaceans in ozonated sea water for long periods (several weeks to several months) is a new research field, requiring innovative technologies to be developed and further experimental trials to be performed on its effects on moulting.

In the penaeid shrimp industry it is common practice to disinfect newly hatched nauplii according to the following protocol (OIE, 2003):

(1) rinse newly hatched nauplii with running sea water for 1 to 2 minutes;
(2) dip them in a 400 ppm formalin solution for 30 to 60 seconds;
(3) dip them again in iodophor (a solution with 0.1 ppm of iodine) for 1 minute; and
(4) rinse the nauplii in running sea water for 3 to 5 minutes before transferring them to disinfected larval rearing tanks.

Ritar *et al*. (2006) disinfected the surface of newly hatched spiny lobster phyllosomas by placing them for 30 minutes in sea water with a final concentration of 25 ppm formaldehyde. After this procedure, the authors selected only the larvae swimming at the surface of the container where the disinfection process was performed. So far, no study has ever reported any specific protocol to disinfect newly hatched marine ornamental shrimp larvae. Therefore, it is advisable that such disinfection protocols be rapidly established, since they can significantly decrease the risk of unwanted microbiological contamination. Nevertheless, it must be kept in mind that the tolerance level displayed by penaeid shrimp nauplii, as well as by spiny lobster phyllosomas, may be significantly different from that of newly hatched zoeae of ornamental shrimps, which may even present species-specific tolerance levels to disinfectants.

The use of UV radiation to control microbiological loads on larval culture systems is also a viable alternative. However, before a UV dose can reach target organisms, it must be transmissible through the water. Suitable UV transmittance can only be achieved if water turbidity remains within certain limits. Additionally, to achieve UV disinfection for a particular group

of microorganisms a minimum UV dose must be maintained through a certain period of time. This dose depends on UV lamp type and power, since a decline of at least 40% in bulb intensity (during a typical 12-month lamp life) can be expected. UV lamps are usually contained within quartz sleeves, allowing them to be submerged in flowing water. Quartz sleeves must be regularly cleaned because the accumulation of detritus may significantly affect transmittance. Neglecting routine inspection and cleaning of UV filters will always result in poorer performance, with suboptimal levels of disinfection that may allow the proliferation of unwanted microorganisms. Applying UV irradiation for disinfection can be less costly and complex than ozonation, presenting the additional advantage of not generating toxic residuals (Summerfelt, 2003). Despite this, ozonation is sometimes preferred over UV filtration since, in addition to inactivating pathogens, it allows the oxidation of organic wastes (including the 'yellowing' agents affecting water coloration) and improves the performance of other water treatment units.

The abusive use of antibiotics in aquaculture to control microorganisms may have serious consequences, namely the development of drug-resistant strains of pathogens (Weston, 1996). The prophylactic application of chemotherapeutic agents may also promote such negative consequences (Brown, 1989). Although marine ornamental shrimps are not destined for human consumption, the use of antibiotics is still highly discouraged for the reasons described above. From a marketing perspective, the negative image associated with the use of antibiotics in aquaculture can be highly disadvantageous for any enterprise culturing marine ornamental species. Since microbial resistance to antibiotics seems to be rapidly increasing (Khachatourians, 1998), the use of non-pathogenic bacteria as probiotic control agents has started to be seriously addressed in aquaculture (Verschuere *et al.*, 2000a; Vaseeharan & Ramasamy, 2003). Parker (1974) defined probiotics as 'organisms and substances which contribute to intestinal microbial balance', while Fuller (1989) restricted the definition to 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. Another definition of probiotics has been proposed by Coeuret *et al.* (2004) as 'living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition'.

Since the objective of using probiotics in culture trials is to produce a proper balance between beneficial microorganisms and the pathogenic microflora of digestive organs and their environment, Farzanfar (2006) highlights the following properties for a successful probiotic:

(1) antagonism to pathogens, a property of probiotic bacteria (Verschuere *et al.*, 2000a), and stimulation of the immunity of its host (Irianto & Austin, 2002);
(2) it must benefit the host animal in some way, such as serving as a growth promoter or protecting the host against bacterial pathogens, with strains producing beneficial substances (e.g. vitamins: Fuller, 1992);

(3) it must have the ability to survive and/or colonize the gut of its host species by adhesion (Verschuere *et al*., 2000a);
(4) it must display high adhesion potential, since this is considered a prerequisite for successful colonization, and be stable under storage and field conditions (Fuller, 1992);
(5) it must be non-pathogenic and non-toxic, avoiding deleterious effects when administered to cultured organisms (Fuller, 1992); and
(6) it must be of animal origin, since these bacteria have a better chance of outcompeting resident ones and establishing in significant numbers in their new host (Rengpipat *et al*., 2003; Alvandi *et al*., 2004).

Gram-positive bacteria, such as *Bacillus*, offer a viable alternative to antibiotic use for penaeid shrimp farming, since these microorganisms are commonly found in marine sediments and are naturally ingested by shrimps that feed in or on the sediments (Farzanfar, 2006). Whether *Bacillus* may also be successfully employed for marine ornamental shrimp culture remains an open question. These microorganisms present the advantage of being unlikely to use genes for antibiotic resistance or virulence, a problem commonly faced when employing *Vibrio*, *Pseudomonas* or other related Gram-negative bacteria (Moriarty, 1999). A clear example of the successful employment of *Bacillus* as probiotics for shrimp culture is described by Vaseeharan & Ramasamy (2003), reporting the antagonistic effect of *B. subtilis* BT23 against pathogenic *Vibrio* in *P. monodon*. In practical terms, this antagonistic effect has been reflected in a reduction of over 90% in accumulated mortality.

Nowadays, it is widely accepted that understanding the microbial community associated with larval-rearing systems is vital for the development of effective strategies for microbial management (Skjermo & Vadstein, 1999; Bourne *et al*., 2004). According to Payne *et al*. (2006), the effective management of the microbial community within a larval rearing system must address the composition and dynamics of these organisms within the water column, cultured larvae, biofilms and live prey. Deleterious bacteria may be displaced from culture systems through the addition of selected bacterial species (Moriarty, 1999), with single strains being less effective than mixed cultures of probiotics (Farzanfar, 2006). The continuous water intake of cultured larvae increases the influence of the microbiological composition of the surrounding medium (Gatesoupe, 1999), since larvae may ingest bacteria by grazing on or filtering the suspended particles. In penaeid shrimp culture, it has been suggested that probiotics may play a more effective role when applied to naupliar stages, before the digestive tract and immune systems are fully developed (Rengpipat *et al*., 2003). This approach may induce a faster change in the intestinal microbiota of cultured larvae, mainly due to the inclusion of microorganisms coming from surrounding water and food particles (Vadstein, 1997). Stenopodidean and caridean shrimp larvae already hatch as fully developed zoeae and not in the naupliar stage

like penaeid shrimps. In this way, the best approach to maximize the use of probiotics during larval culture trials still needs to be investigated, since it may differ from the methods commonly employed for penaeid shrimps.

An interesting approach that may help in finding suitable probiotics for marine ornamental shrimp larval culture will be to identify the microbiological community associated with these larvae in the wild. However, this will not be an easy task since it entirely relies on plankton samples, whose composition is totally unpredictable.

7.2.4 Importance of water temperature, larval density, and photoperiod

During culture trials it is not unusual to expose decapod larvae to a variety of potential stress factors, including high stocking densities and suboptimal water conditions. Inadequate water temperatures, excessive intraspecific interactions promoted by high rearing densities and unnatural photoperiods (e.g. 24 hours of light or 24 hours of darkness) may increase larval susceptibility to the action of pathogens (Takahashi *et al.*, 1995).

The inverse relationship between rearing temperature and the duration of larval stage has been well documented for several decapod species (Anger, 2001). This relation has been commonly employed in commercial larval culture, decreasing the duration of the larval period by increasing water temperature. A good example of such a practice is provided by the work of Calado *et al.* (2005a) addressing the larval culture of *L. seticaudata*, with cultured larvae raised at 20°C settling on average 32 days post-hatching, while those cultured at 26°C settled 19 days post-hatching. High rearing temperatures can significantly increase the risks of larval mortality, since they induce higher metabolic rates and may amplify eventual nutritional deficiencies experienced by cultured larvae (Knowlton, 1974). However, as documented by Calado *et al.* (2005a), if suitable larval diets are employed, the period of larval development can be significantly shortened and still achieve high survival rates. Marine ornamental shrimp larvae are commonly cultured at temperatures ranging from 25 to 29°C. However, when developing culture protocols and establishing suitable dietary regimes, it is advisable to work at 25–26°C. This approach may allow culture bottlenecks to be progressively overcome, namely those related to larval nutrition, without promoting higher mortality. This cautious approach commonly allows a significant number of larvae to reach a critical zoeal stage, or even metamorphosis, providing enough biological material to perform biochemical and physiological studies. After establishing a satisfactory culture protocol, water temperature may be increased to higher values (28–29°C) to shorten larval duration, and other culture parameters can be adjusted in order to optimize survival.

Rearing densities commonly employed in marine ornamental shrimp larval culture are usually lower than 50 larvae per liter. One of the main reasons for the use of such low rearing densities is the large size reached by late zoeal stages, namely those of *Lysmata* and *Stenopus*. The use of high rearing densities at later zoeal stages commonly promotes the occurrence of agonistic interactions between cultured larvae. At higher densities, it is not unusual to record late zoeae displaying a single eye, or even missing both eyes, clearly indicating the occurrence of cannibalism. Another reason to avoid high culture densities is related to the physical constraints of recirculated systems commonly employed to culture these larvae. It is known that feeding rates consistently increase during larval development (e.g. Tong *et al*., 1997; Zhang *et al*., 1998b; Narciso & Morais, 2001) and that despite decapod zoeal stages being generally considered good swimmers, true hunting behaviors (involving prey chasing) are not displayed (Gonor & Gonor, 1973; Epelbaum & Borisov, 2006). Therefore, larvae entirely rely on chance encounters with dietary prey, a type of feeding behavior described by Berkes (1975) as 'encounter feeding'. Because of the clogging of mesh screens employed in recirculated culture tanks, the highest density of dietary prey that can be provided is limited to a maximum value, which is commonly inferior to the feeding requirements displayed by cultured larvae. Calado *et al*. (2005a) reported that a significantly higher number of *L. seticaudata* larvae displayed terminal additive moulting when cultured at 40 larvae per liter than at lower densities (10 and 20 larvae per liter). The authors attributed these results to the suboptimal level of dietary prey available for larvae being cultured at higher temperatures. A potential solution for the problem described above could be the regular supply of dietary prey during the day, ensuring that cultured larvae always have an adequate density of food items available. However, this is a tedious operation, which does not eliminate the risks of clogging the mesh screen filters present in larval culture tanks.

The effect of different light regimes has already been investigated for several decapod larvae, commonly indicating species-specific results. Minagawa (1994) reported lower prey consumption rates for newly hatched larvae of the red frog crab *Ranina ranina* (Linnaeus, 1758) kept in darkness than when kept with 24 hours of light. However, Harvey & Epifanio (1997) did not record any significant effect of light regimes on food intake displayed by newly hatched larvae of the common mud crab *Panopeus herbstii* Milne Edwards, 1834. Unlike fish larvae, which are visual feeders (Blaxter, 1986), decapod crustacean larvae do not seem to use sight to locate prey (Epelbaum & Borisov, 2006). In this way, light may play a minor role in shrimp larval culture when compared with fish larval rearing. None the less, light may promote more intense swimming activity and consequently affect prey ingestion indirectly by promoting more 'chance encounters' with potential prey (Strathmann, 1987). Concerning marine ornamental shrimp larvae, the only study available is a preliminary trial comparing the effect of three different photoperiods (24, 12 and 0 h of

light) on the feeding rate of newly hatched larvae of four *Lysmata* species (*L. amboinensis*, *L. ankeri*, *L. debelius* and *L. seticaudata*). There is no evident benefit in exposing newly hatched *Lysmata* larvae to 'extreme' photoperiods (24 or 0 h of light), since larval feeding rates are not significantly influenced (Calado, unpubl. obs.). Studies by Knowlton (1974) and Mikami & Greenwood (1997) have even reported the occurrence of higher mortality in larvae raised either in total darkness or with 24 h of light. Large numbers of *Lysmata seticaudata* larvae have already been successfully raised in total darkness, being daily exposed to light for only 1 hour while performing routine cleaning and feeding. Dietary prey (*Artemia* nauplii) and larvae cultured under these conditions were recorded to be more evenly distributed through the tank water column, eliminating the typical 'larval crowding effect' promoted by light at the water surface (Calado, unpubl. obs.). The fact that late zoeal stages of some marine ornamental shrimps (e.g. *Lysmata*) reverse their behavioral response to light, becoming negatively phototactic (A.L. Rhyne, pers. comm.), may also prove to be a problem during larval culture. During light hours, cultured larvae will seek refuge in shaded areas and their larval prey (commonly displaying positive phototactic behavior) will remain in the most well illuminated portions of the culture tank. In extreme situations this may promote the occurrence of nutritional stress as a result of suboptimal feeding rates. Another important aspect that needs to be addressed during the culture of newly hatched alpheid (pistol shrimps) and rhynchocinetid (dancing shrimps) larvae is their strong positive phototactic behavior. In the presence of light, this behavior commonly leads newly hatched larvae to swim rapidly towards the water surface of the culture tank and become trapped in the air/water interface. These larvae are unable to oppose the existing superficial tension, remain trapped at the water surface, are unable to actively chase dietary prey items and invariably starve to death. A simple solution to prevent this behavior is to culture at least the first zoeal stage of alpheid and rhynchocinetid larvae in total darkness, since the second zoeal stage no longer becomes trapped at the water surface.

The apparent benefits of raising marine ornamental shrimp larvae in darkness still need be confirmed through additional culture trials, with emphasis on the occurrence of species-specific deleterious effects on late zoeal and post-larval quality.

7.2.5 Settlement cues

The earlier trials addressing commercial-scale culture of *Stenopus* and *Lysmata* considered that this goal would only be achieved if the specific stimuli that promoted the settlement of late-stage larvae could be identified (Fletcher *et al.*, 1995). Later work on these and other marine ornamental shrimp genera continues to suggest that the long larval periods commonly displayed by cultured

larvae could be attributed to the absence of appropriate settlement cues during captive rearing (Calado *et al*., 2003b). However, recent laboratory evidence recording the existence of secondary facultative lecithotrophy in *L. seticaudata* (Calado *et al*., 2007b), seems to indicate that nutritional issues must be responsible for the prolonged duration of the larval period in marine ornamental caridean and stenopodidean species. Apparently, only after reaching a critical level of stored energy will the last zoeal stage moult to the megalopa. The same rationale has already been used to explain the extended periods of larval development recorded for spiny lobsters (Lemmens, 1994, 1995; McWilliam & Phillips, 1997). Metamorphosis delay due to suboptimal nutrition was for long considered to be a laboratory artefact resulting mainly from the use of unsuitable larval diets during culture trials (Gore, 1985). However, field evidence indicates that wild decapod larvae also postpone metamorphosis (Gebauer *et al*., 2003). Gurney & Lebour (1941) have described how the last zoeal stages of the dancing shrimp *Cinetorhynchus rigens*, collected from plankton samples, displayed similar morphological features but significantly different body size. None the less, both different-sized late zoeal stages were able to successfully moult to megalopa. The considerably larger size displayed by these wild larvae indicates that, under natural conditions, late zoeal stages can also delay metamorphosis by extending their larval development.

Despite the relevance of nutritional aspects, settlement cues also play a crucial role for the successful settlement of certain marine ornamental shrimps. The most well documented examples are those of partner shrimps *Periclimenes*. Goy (1990) and Calado *et al.* (2004) have clearly demonstrated how release of chemical cues by the host anemones of different *Periclimenes* species promoted shorter larval durations and higher survival during culture trials. However, direct contact by host anemone and cultured larvae invariably resulted in the latter being stung to death, revealing that the tolerance displayed by the host anemone towards their symbiotic shrimps is only acquired after metamorphosis. For this reason, when culturing *Periclimenes*, or any other shrimps displaying symbiotic behavior, the host species must never be placed inside the larval culture tank. The host species should preferably be placed in an empty container, such as a header tank, from which water is allowed to flow to the culture vessels. This practical approach allows chemical cues to be carried with inflowing water from the host species to the cultured tank, without any potential risk for cultured larvae. It is important that water flowing from the container holding the host species to the larval culture tank is not submitted to any type of chemical filtration, since this could result in the removal or modification of the chemical cue molecules inducing settlement.

Several studies have shown that specific features displayed by sediments of the adult habitat can act as effective cues to induce metamorphosis in late-stage larvae of decapod crustaceans (Botero & Atema, 1982; Christy, 1989; Weber & Epifanio, 1996; O'Connor & Judge, 1997; Gebauer *et al*., 1998; Van

Montfrans *et al.*, 2003). This type of settlement cue may be important to trigger the metamorphosis of alpheid shrimps which share their burrows with fishes. However, this hypothesis has never been experimentally addressed. According to Yanagisawa (1984), it appears that only after a juvenile shrimp has constructed a burrow will a juvenile fish seek shelter in it and establish their associative bond. In this way, cues released by the presence of the partner fish species may be less effective than sediment cues to trigger metamorphosis. When using sediment to promote metamorphosis in larvae being cultured in captivity, a similar technical approach to that described above for host species should be followed. As an alternative it may be possible to suspend in the rearing tank small containers (e.g. Petri dishes) containing sediment. It is important to ensure that water flow dynamics is not negatively influenced by the presence of these structures inside the culture tanks and that the sediment is not disturbed during the settlement period, since it may clog the mesh screens placed in the tank outflow and negatively affect water quality.

Odours released by adult conspecifics are also known to trigger metamorphosis in several decapod species (Jensen, 1989, 1991; Harvey, 1996; O'Connor & Gregg, 1998; Rodríguez & Epifanio, 2000; Gebauer *et al.*, 2002). It has been suggested that gregarious settling with adult conspecifics may enhance post-larval survival, with such clusters acting as important mechanisms to avoid predators (Jensen, 1989). Curiously, the potential of conspecifics' cues to trigger metamorphosis in marine ornamental shrimp larvae has never been properly investigated. Species living in stable mated pairs (e.g. *Hymenocera*, pair-living *Lysmata* and *Stenopus*) would certainly benefit from such adaptation, since they commonly occur in the wild at low densities and would considerably increase their chances of finding a conspecific to pair with. Gregarious species occurring in large groups of individuals (e.g. crowd-living *Lysmata*, *Rhynchocinetes* and *Cinetorhynchus*) may also benefit from settling in a favourable environment near conspecifics. When testing the influence of conspecifics' cues to promote metamorphosis in larvae being cultured in captivity, it is not advisable to place adult or juvenile conspecifics inside the rearing tank since they will certainly cannibalize the larvae. As previously described for testing settlement cues released by hosts of symbiotic species, conspecifics can also be placed in the container supplying the inflowing water to culture tanks. In conclusion, researchers addressing the larval culture of marine ornamental shrimps should start evaluating the role played by the structural and textural features of the substratum occupied by adults in triggering metamorphosis (Aziz & Greenwood, 1982; Day & Lawton, 1988; Butler & Herrnkind, 1991). Additionally, by understanding the role in the settlement process of biogenic and water-soluble compounds released by conspecifics, host species or dietary prey (Welch *et al.*, 1997; Fitzgerald *et al.*, 1998; Gebauer *et al.*, 2002), significant breakthroughs for the culture of marine ornamental shrimps can be achieved. The role of microbial biofilms, as well as the

existence of positive or negative synergetic effects between different types of settlement cues (Forward *et al*., 2001), must also be addressed in future studies.

7.3 Larval feeds and nutrition

In the wild, decapod crustacean larvae have access to a wide variety of potential food items: dissolved organic matter, microorganisms belonging to picoplankton (size inferior to 2 μm) and nanoplankton (between 2 and 20 μm), detritus and faecal pellets, phytoplankton (namely microplankton with sizes between 20 and 200 μm), microzooplankton (between 20 and 200 μm), mesozooplankton (between 200 and 2000 μm) and macrozooplankton (greater than 2000 μm) (Anger, 2001). It is obvious that these dietary items display high quantitative and qualitative differences and allow developing larvae to select the most suitable group of prey during each zoeal stage. The large amount of different prey naturally available to developing larvae has favoured the evolution of selective feeding mechanisms in particle feeders (Strathmann, 1987). This degree of selectivity is variable in larval development, with Jones *et al.* (1997b) highlighting that later larval stages of caridean shrimps accept a wider range of particle food types. Anger (2001) suggested that early decapod larval stages actively select small dietary prey whereas later stages seem to exhibit a preference for larger items.

Caridean and stenopodidean larvae are generally considered to be carnivorous (Le Vay *et al*., 2001). However, as already recorded for other decapod larvae, their survival and growth may depend on the consumption of additional alternative prey, namely phytoplankton, protists and eventually detritus, at least in early zoeal stages (Sulkin & van Heukelem, 1980; Sulkin & McKeen, 1999; Hinz *et al*., 2001; Perez & Sulkin, 2005). In contrast, late larval stages of some caridean and stenopodidean shrimps (e.g. *Lysmata* and *Stenopus*) can attain such large sizes that they are among the few decapod larvae that can capture and ingest macrozooplankton or other large dietary items (such as minced squid, shrimp or chopped mussel) (Figure 7.3).

Most studies addressing larval feeding of decapod crustaceans has focused on those species currently being used for human consumption. Usually, these studies are biased towards aquaculture-related aspects, namely the establishment of suitable culture protocols using domesticated species of phytoplankton and zooplankton as live prey, as well as the development of inert artificial feeds. Numerous technical constraints still impede realistic studies to address nutritional aspects of decapod larvae in the wild (Anger, 2001). The impossibility of simulating the quality, quantity, diversity and spatial distribution of dietary prey when performing captive culture trials of decapod larvae may limit our knowledge on the most suitable dietary items throughout larval development. The few existing studies addressing the feeding habits of caridean shrimp larvae in the laboratory using wild zooplankton (Bergström,

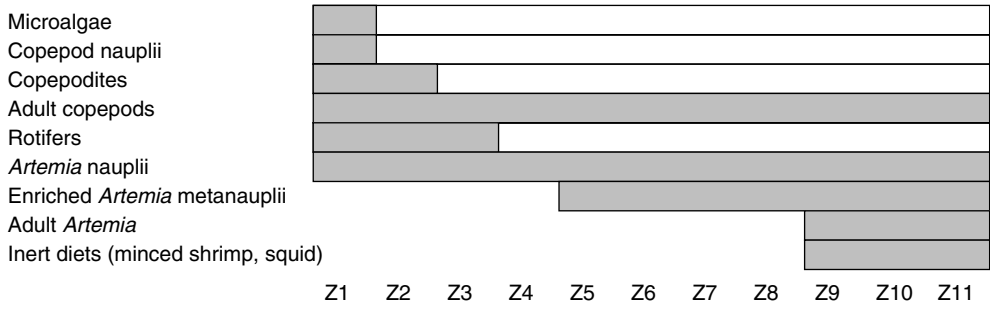


Figure 7.3 Theoretical feeding scheme for a marine ornamental shrimp displaying eleven zoeal stages (grey bars); potential feeding regime when employing a mesocosm (inert diets are not usually employed in this culture approach) (white bars).

2000; Harvey & Morrier, 2003) confirmed the shift in prey preference during larval development and an exponential increase in predation rate among successive larval stages.

The lipid content of wild-caught pelagic organisms has been successfully used to identify the diet of pelagic crustacean predators (Phleger *et al*., 1998; Virtue *et al*., 2000; Nelson *et al*., 2001). Jeffs *et al*. (2004) were able to provide valuable information on the complex feeding habits of spiny lobster larvae in the wild through the comparison of signature lipid profiles of a wide range of wild zooplankton and those of phyllosoma larvae at different developmental stages. A molecular approach adopted by Suzuki *et al*. (2006) to investigate the natural diets of palinurid and scyllarid lobster phyllosoma was also highly successful. Emerging techniques of genetic 'fingerprinting' may allow researchers to further investigate feeding dynamics during different seasons and through larval development of decapod species, helping to improve current culture protocols (Jeffs, 2007).

The use of similar approaches to study the larvae of marine ornamental shrimps in the wild can also provide valuable information to optimize current larval culture methodologies. However, because of their frail morphology, transparent-like appearance, pelagic distribution and low natural density, the collection of enough biological material for experimental observations and analysis will not be easily achieved.

The most commonly used dietary items in marine decapod larval culture are described below, highlighting which ones have already been successfully used to raise marine ornamental shrimps (Table 7.1).

7.3.1 Microalgae

Microalgae have been largely employed as a live feed in the larval culture of decapod crustaceans, with emphasis on penaeid shrimps, as well as in the culture of various other zooplankton species also used in aquaculture

Table 7.1 Marine ornamental shrimp species already cultured in captivity, with details on the minimum number of days recorded to metamorphosis, larval diets employed during culture and special remarks on the culture conditions employed

Family	Species	Days to metamorphosis	Larval diets	Special remarks	Reference
Stenopodidae	*Stenopus hispidus*	120 days	Microalgae, rotifers, newly hatched *Artemia* nauplii	None	Fletcher *et al.*, 1995
	Stenopus scutellatus	43 days	Newly hatched *Artemia* nauplii	Larvae were hatched from an embryo clutch spawned in the wild	Zhang *et al.*, 1997b
Hippolytidae	*Lysmata amboinensis*	58 days	Microalgae, rotifers, newly hatched *Artemia* nauplii	None	Fletcher *et al.*, 1995
	Lysmata debelius	75 days	Rotifers, newly hatched *Artemia*, puréed shrimp muscle	The authors highlight the importance of feeding puréed shrimp muscle to later zoeal stages	Palmtag & Holt, 2001, 2007
	Lysmata 'rathbunae' [1]	19 days	Rotifers, newly hatched *Artemia*	None	Wittenrich, 2002
	Lysmata seticaudata	19 days	Newly hatched *Artemia* nauplii	None	Calado *et al.*, 2005a
	Saron marmoratus	10 days? [2]	Live microplankton collected daily	None	Sankolli & Kewalramani, 1962
	Thor amboinensis	46 days	Sea urchin blastulae, rotifers and newly hatched *Artemia* nauplii	Exposure to anemone exudates revealed to be unnecessary during larval culture	Sarver, 1979

Hymenoceridae	*Hymenocera picta*	Approximately 35 days	Microalgae, rotifers, newly hatched *Artemia* nauplii, minced crab and bivalves and <275 μm wild zooplankton	Despite the wide number of tested larval prey, the author considered *Artemia* nauplii alone to be a suitable diet for successful larval culture	Fiedler, 1994
		Approximately 28 days	Microalgae, rotifers, copepods and newly hatched *Artemia* nauplii	The author advocates that rearing success is largely enhanced with the use of copepods	Kraul, 1999
Palaemonidae	*Periclimenes pedersoni*	28 days	Newly hatched and juvenile *Artemia*	Continuous exposure to exudates of the anemone *Bartholomea annulata*	Goy, 1990
	Periclimenes yucatanicus	29 days	Newly hatched and juvenile *Artemia*	Continuous exposure to exudates of the anemone *Condylactis gigantea*	Goy, 1990

[1] Due to the recent description of the *wurdemanni* species complex by Rhyne & Lin (2006) it is not possible to accurately determine the true identity of most species employed in earlier culture trials.
[2] The true larval period of this species may have been underestimated by Sankolli & Kewalramani (1962).

as live prey (Brown *et al*., 1997). In recent decades the potential of several hundred microalgae species for aquaculture purposes has been evaluated. However, only a limited number of species (<25) have become widely used in commercial-scale cultures. According to Brown (2002), microalgae must have the following features to be successfully employed in aquaculture. They must:

(1) display an appropriate size for ingestion (from 1 to 15 μm);
(2) be readily digested;
(3) display high growth rates to allow mass culture, while tolerating moderate fluctuations in temperature, light and nutrient levels; and
(4) display a nutritional profile suitable to fulfil the requirements of cultured species.

The use of microalgae, namely *Skeletonema* spp. and *Chaetoceros* spp., plays a crucial role in the successful culture of the early filter-feeding larval stages of penaeid shrimps (Dall *et al*., 1990; Preston *et al*., 1992; Ronquillo & Saisho, 1997). However, newly hatched caridean and stenopodidean larvae retain only a limited ability to filter feed on small particles (Strathmann, 1987). A diet of microalgae alone has proven to be insufficient to adequately feed marine ornamental shrimp larvae [e.g. Zhang *et al.* (1997b) for *Stenopus* and Zhang *et al.* (1998c) for *Lysmata*]. None the less, supplying microalgae during the culture of the first larval stages, and even during later stages, may prove to be an advantageous practice. This approach has already been advocated by Simões *et al.* (2002) in order to minimize the effects of starvation on newly hatched *L. debelius* larvae since the peristaltic movement of the hindgut seems to indicate that these larvae are already able to swallow water and consequently suspended food particles. In the wild, phytoplankton seems to be an important food source for larval decapods, as confirmed by the presence of microalgae in their guts and faeces (Lebour, 1922; Anger, 2001). The presence of microalgae pigments has also been recorded in decapod larvae through analytical techniques (Meyer-Harms & Meyer, 1993; Harms *et al*., 1994). As recorded for fish larvae by Naas *et al.* (1992), the presence of microalgae may modulate the light conditions in larval culture tanks and improve the feeding behavior of shrimp larvae. The use of the 'pseudo-green' water method, characterized by the frequent addition of phytoplankton to larval rearing tanks, may also be highly beneficial during early larval culture, when larvae are more vulnerable to nutritional stress (Papandroulakis *et al*., 2001). Alabi *et al.* (1999) and Brown (2002) note that the addition of microalgae to culture tanks may benefit cultured larvae through the excretion of vitamins, or other growth-promoting substances, and through their probiotic role as selective bactericides.

It is known that HUFA-rich microalgae can be effectively used to improve the nutritional value of zooplankton, namely their DHA levels, before they are used as live prey in culture trials (Nichols *et al*., 1989; Fernández-Reiriz &

Labarta, 1996; Palmtag *et al*., 2006). The addition of *Isochrysis*, *Nannochloropsis*, *Pavlova* and *Tetraselmis* has proven to be very effective in the culture and/or enrichment of rotifers and *Artemia*, improving or prolonging the nutritional quality of larval prey provided to cultured larvae. Marques *et al*. (2006) have also confirmed that the use of particular microalgae (e.g. *Dunaliella*) may confer full protection to gnotobiotic *Artemia* nauplii against pathogenic *Vibrio*.

The use of microalgae in recirculated larval culture systems for larval decapods can present some technical limitations, since water renewal rates commonly employed will rapidly remove the microalgae from culture tanks. Additionally, the microalgae removed will certainly be damaged by filtration systems and pumps, leading to a sharp decrease in water quality and threatening the survival of cultured larvae. A possible solution to overcome these technical limitations will be to run the larval culture tanks as an open system, regularly adding a certain amount of microalgae to keep algal concentrations at an optimum level. Another option will be to run larval culture tanks as a static system with daily renewals of at least 50% of the water volume. However, this option presents the risk of cultured larvae being exposed to extremely low levels of dissolved oxygen, which in a worst case scenario may lead to mass larval mortality. Kittaka (1994b) described an 'ecosystem culture method' for spiny lobster phyllosoma, which may also be employed for the culture of the early larval stages of marine ornamental shrimps. In such systems, the water is recirculated between the upwelling larval culture tanks and a large culture container of microalgae. According to Kittaka (1994b,c), the use of microalgae, along with regular water changes every 2 weeks, enables ammonia to be kept below detectable levels. This methodology assured good water quality through the entire duration of culture trials, even when providing dietary items that rapidly polluted culture water such as small pieces of mussel gonad.

Since the production of fresh microalgae requires special facilities, daily maintenance and skilled labour, and represents a significant part of the costs associated with hatchery operations (Jeffrey & Garland, 1987), suitable alternatives have started to be evaluated in recent years. Spray dried, sun dried, flocculated and centrifuged concentrates of microalgae, either fresh or refrigerated, have been successfully used to culture penaeid shrimps, totally, or at least partially, replacing the need for live microalgae (Biedenbach *et al*., 1990; Millamena *et al*., 1990; D'Souza *et al*., 2000, 2002). Future studies must also address the suitability of these alternatives to live microalgae in the larval culture of marine ornamental shrimps.

7.3.2 Rotifers

The marine rotifer *Brachionus plicatilis* has rapidly become an important starter live feed for larval culture of decapod crustaceans (Dhert *et al*., 1997). This

larval prey ranges in lorica size from 100 to 340 μm (Fukusho, 1989). Rotifers with a high nutritional profile can be produced nowadays, although their quality may sometimes still be suboptimal. The major cause of these quality issues is hygiene problems, which have led researchers to develop suitable methodologies to address microbiological aspects involved in rotifer culture (Rombaut *et al*., 1999). The most common problems affecting the production of rotifers are (Yoshimura *et al*., 1996):

(1) the unpredictability of their mass production;
(2) management and harvest problems when working with large rotifer populations; and
(3) the challenging task of producing clean rotifers, free from floccules and without deleterious microorganisms.

The nutritional profile of cultured rotifers needs to be corrected and/or improved to fulfil the needs of cultured larvae. Rotifer enrichment can be easily achieved through the use of selected microalgae. Fatty acid contents of rotifers can be boosted by using good quality live microalgae as food. The EPA (20:5*n*-3) and DHA (22:6*n*-3) levels of rotifers can be significantly increased by using the microalgae *Nannochloropsis* for EPA enrichment (e.g. Watanabe *et al*., 1983; Sukenik *et al*., 1993) or *Isochrysis* and *Rhodomonas* for DHA boosting (Lubzens *et al*., 1985; Ben-Amotz *et al*., 1987; Sukenik & Wahnon, 1991). The main reason for the success of this procedure is that rotifers are easily able to incorporate these essential fatty acids in a few hours. Commercial products currently available also allow the improvement of the lipid, vitamin and protein levels of rotifers. Two approaches are usually followed when enriching rotifers with these products (Dhert *et al*., 1997):

(1) lipid and vitamin content of rotifers is adjusted less than 8 hours before feeding them to cultured larvae (short-term enrichment); and
(2) rotifers are fed a complete diet for more than 24 h (long-term enrichment).

During long-term enrichment the complete body of rotifers, not just the digestive tract, is modified to a composition similar to that of the diet on which the rotifers were grown. Rotifers fed according to this procedure are nutritionally more stable, losing their reserves more slowly than those placed under short-term enrichment. The success of commercial enrichment products is mainly related to the fact that rotifers do not selectively take up or catabolize their components, namely HUFA (such as EPA and DHA).

In larval decapods it is common to record a shift in larval prey preference during development, with early zoeal stages preferring smaller food items (e.g. rotifers) whereas later stages switch to larger prey (e.g. *Artemia* nauplii) (McConaugha *et al*., 1991). However, larger stages continue to ingest

smaller prey if these are available (Harvey & Epifanio, 1997), with decapod larvae becoming increasingly selective in the presence of higher prey densities (Paffenhöffer & Knowles, 1978; Yúfera *et al*., 1984). Prey discrimination in larval decapods does not seem to be visual (Strathmann, 1987): cultured larvae presented a variety of prey are able to discriminate them either by size and/or swimming behavior (Harvey & Epifanio, 1997).

A diet based entirely on rotifers is not adequate to successfully raise marine ornamental shrimps to metamorphosis (Zhang *et al*., 1997b, 1998c). However, feeding rotifers to early larval stages of marine ornamental shrimps (up to zoea III) can be important to minimize early larval mortality and successfully culture the larvae until metamorphosis (Palmtag & Holt, 2001, 2006). Similar approaches have proved to be critical for the successful larval culture of zoeal crabs (Sulkin & Epifanio, 1975; Sulkin, 1978; Suprayudi *et al*., 2002; Ruscoe *et al*., 2004). The first zoeal stage of ornamental shrimps differs morphologically, even among species within the same genus (see Calado *et al*., 2004, for examples in the genus *Lysmata*). Newly hatched larvae of some shrimp species can display less developed mouth parts (e.g. maxillipeds), limiting their ability to capture large prey items (e.g. *Artemia* nauplii and enriched metanauplii). These larvae can capture and ingest smaller prey, such as rotifers, more readily and efficiently, allowing them to maximize the budget between the energy they get from digesting captured prey and the energy spent on capturing them. Since mouthparts grow in size and complexity during larval development, successive zoeal stages become able to capture larger food particles and gradually shift their preference towards bigger prey (Anger, 2001).

7.3.3 *Artemia*

Although *Artemia* is not a natural prey for marine larval decapods, its use as live food in the captive culture of these organisms has become a widespread and successful practice in recent decades. Apart from its nutritional value, *Artemia*'s popularity is mainly because it is an off-the-shelf food as its dormant cysts can be stored for long periods in cans (Sorgeloos *et al*., 1998). Good grade *Artemia* cysts require only 24 h (or less) of incubation before hatching as nauplii, and are the most convenient and certainly one of the least labour-intensive live foods available for the culture of larval crustaceans. Newly hatched *Artemia* nauplii can measure between 422 and 517 μm in length (Léger *et al*., 1986). Newly hatched *Artemia* nauplii are unable to feed, although they readily consume their own energetic reserves, and moult to their second larval stage (metanauplii) 6 to 8 hours later (Benijts *et al*., 1976). When culturing larval decapods it is important to offer newly hatched nauplii to the larvae instead of starved metanauplii, since these display nearly twice the length of newly hatched nauplii and are faster swimmers. Starved metanauplii are thus a dietary item with inferior quality, displaying significantly lower levels of

free amino acids and energy (Léger *et al.*, 1986). Cultured larvae that are fed starved metanauplii will always display poorer survival rates, since the energy uptake they will get from digesting starved metanauplii will not be sufficient to compensate for the higher energetic investment required to capture these larger and fast swimming prey. The deleterious effects of this practice have already been verified by Calado *et al.* (2005a) when feeding starved metanauplii to the zoeal stages of *L. seticaudata*. The daily removal of 'old' metanauplii, while also providing fresh, newly hatched nauplii, allows cultured larvae to have continuous access to good quality prey during their entire development (Calado *et al.*, 2003a). This routine may not be necessary if microalgae are also present in the larval culture tank, since uneaten *Artemia* metanauplii may ingest them and regain nutritional value.

An interesting approach suggested by Mock *et al.* (1980) to facilitate the capture of larval prey by cultured larvae is to provide them with cold-stored newly hatched nauplii. This procedure reduces nauplii swimming activity, promotes much higher food uptake and may allow poorly developed newly hatched larvae to successfully capture such prey. As the size of *Artemia* nauplii can vary significantly according to their geographical origin, another alternative for feeding poorly developed newly hatched marine ornamental shrimp larvae is to choose an *Artemia* strain with small cyst diameter (under 230 μm) (Vanhaecke & Sorgeloos, 1980). Using small-strain *Artemia* nauplii may eventually eliminate the need to provide rotifers, or other small prey, during early zoeal stages.

The nutritional value of *Artemia* is known to display geographical and seasonal variations (Sorgeloos *et al.*, 1998). However, it may be corrected through the use of microalgae or other commercial enrichment products. Emulsified lipophilic products are commonly employed to enrich *Artemia*, allowing these live prey to deliver higher amounts of essential nutrients (e.g. HUFA and vitamins) to cultured larvae. However, enrichment techniques display certain limitations, mainly due to the ability of *Artemia* to selectively catabolize some of the enriching nutrients, namely DHA and phospholipids. Whether *Artemia* bioaccumulates the nutrients provided during enrichment in their digestive tracts, or they are assimilated and cumulated in their tissues, will determine for how long enriched *Artemia* can effectively supply higher levels of those specific nutrients to cultured larvae (Sorgeloos *et al.*, 1998). Additionally, it is necessary to ascertain whether enriched *Artemia* selectively catabolizes the nutrients being provided by the enrichment product (Narciso *et al.*, 1999; Smith *et al.*, 2002b). Concerning marine ornamental shrimp culture, enriched *Artemia* metanauplii were successfully employed to culture several species of *Lysmata*, although they do not seem to play a critical role in their culture (Calado *et al.*, 2005a; Palmtag & Holt, 2007). However, the limited number of studies performed that address the advantages of using enriched *Artemia* metanauplii to raise marine ornamental shrimps means it is still impossible to rule out the use of these prey. If marine ornamental shrimps can be cultured without using

enriched metanauplii, larval culture costs will certainly be lower and a more competitive product will be available in the market.

Although *Artemia* is commonly supplied to cultured larvae as newly hatched nauplii or 24-hour-old enriched metanauplii, decapsulated cysts, small juveniles and adult biomass have also been successfully employed as larval diets (Léger *et al*., 1986; Bengtson *et al*., 1991; Kittaka, 1999; Ritar *et al*., 2003). Decapsulated cysts have been used to feed the early larval stages of the ornamental shrimp *L. seticaudata* (Calado *et al*., 2001a), proving that they can be a viable solution to prevent starvation of newly hatched larvae when other larval prey are unavailable. This diet has the advantage of being directly available off the shelf, and cysts with poor hatching quality may still be used as a food source instead of simply being discarded (Sorgeloos *et al*., 1998). The negative aspects of providing decapsulated cysts to cultured larvae are that they rapidly settle in the bottom of culture tanks, becoming less available for planktonic larvae. A possible solution to minimize the settlement of decapsulated cysts may be to increase the water flow inside the culture tank to keep them in suspension. However, such a practice must be carefully monitored in order to prevent physical damage to the frail larvae of marine ornamental shrimps. Late zoeal stages of ornamental shrimps are able to capture juvenile and even adult live *Artemia* (unpubl. obs.). These later larval stages may be exposed to partial starvation when exclusively provided with *Artemia* nauplii, since it is known that large caridean larvae may not be able to ingest these prey in sufficient amounts to provide a positive energy balance (Léger *et al*., 1986). In these situations, supplying juvenile *Artemia* to cultured larvae may allow considerably better larval development and survival to metamorphosis. Anger & Püschel (1986) have shown that juvenile and adult *Artemia* are suitable prey for the large sized carnivorous zoeae of the Norway lobster *Nephrops norvegicus* (Linnaeus, 1758). However, unless live *Artemia* can be harvested from a natural source, more or less complex culture systems (Zmora *et al*., 2002; Dhont & Van Stappen, 2003; Zmora & Shpigel, 2006) must be employed to produce significant numbers of these prey to support commercial-scale culture of ornamental shrimp larvae. In this way, only if future studies prove that the nutritive value of these prey can effectively fulfil the requirements of cultured larvae may this approach be addressed at an economically feasible commercial scale. The use of juvenile or adult *Artemia* collected from the wild must be carefully considered, since it may act as a vector for the contamination of culture systems by pathogens or other deleterious organisms.

Fresh live *Artemia* is the most commonly used form to supply this food during larval culture trials, mainly because of their higher nutritive value. *Artemia* may also be supplied frozen, freeze-dried, in flakes or in other forms of formulated feed (Sorgeloos *et al*., 1998). So far, the use of these alternative forms to supply *Artemia* to marine ornamental shrimp larvae has not been

experimentally evaluated. Potential problems that need to be overcome to successfully employ these food items during larval culture are:

(1) settlement in the tank bottom;
(2) water quality deterioration, namely when using frozen *Artemia*;
(3) lack of food attractants, which results in reduced interest in these food particles on the part of cultured larvae, in opposition to the positive feeding responses produced by live *Artemia*; and
(4) unbalanced nutritional profiles.

The high bacterial load present in newly hatched *Artemia* nauplii may sometimes be a problematic situation for larval culture trials (Lavens & Sorgeloos, 2000). Verschuere *et al.* (1999a, b) proposed the control of the microbial environment of *Artemia* by cyst disinfection, followed by the inoculation of selected bacterial strains into the larval culture system water. Sahul Hameed & Balasubramanian (2000) recommended the disinfection of newly hatched nauplii with formaldehyde before introducing them into the larval rearing systems. Other work advocates the advantages of *Artemia* cultured in gnotobiotic environments and its bioencapsulation using selected microorganisms with probiotic activity (Verschuere *et al.*, 2000b; Marques *et al.*, 2005). Despite the risks of contamination of larval culture systems through the use of newly hatched or enriched *Artemia*, the results achieved in the culture of marine ornamental shrimps when employing these live prey are still difficult to match using any other alternative foods.

7.3.4 Copepods and mesocosms

Copepods are known to be a common dietary item for larval decapods in the wild (Stickney & Perkins, 1981; Anger, 2001). These organisms are natural sources of antioxidants, astaxanthin, and vitamins C and E, and display high levels of HUFAs, namely DHA, EPA and ARA (Støttrup & Jensen, 1990). Laboratory studies have also shown that these larval prey can be successfully used to culture decapods in captivity (Paul *et al.*, 1979; Harvey & Morrier, 2003). In fact, the suitability of the three main copepod orders (Cyclopoida Burmeister, 1834, Calanoida G. O. Sars, 1903, and Harpacticoida G. O. Sars, 1903) as larval diets, mainly for fish species, has been widely addressed (Marcus, 2005), although all orders display advantages and disadvantages in marine aquaculture (Støttrup, 2006). However, the provision of regular and large quantities of copepods is far from being as convenient and reliable as the production of rotifers or *Artemia* nauplii (Støttrup, 2000). Copepod production in extensive and semi-intensive systems tends to be limited to near-shore facilities (Fukusho, 1980; van der Meeren & Naas, 1997; Støttrup *et al.*, 1998), restricting their suitability for enterprises based inland. Additionally, these systems are

highly dependent on the seasonal variations in the abundance of copepods, which limit their regular and reliable supply (Støttrup, 2000). The involuntary introduction in culture systems of certain unwanted organisms, namely cnidarians, is also a potential risk that must be considered when using wild copepods (Helland *et al*., 2003).

Calanoid species are commonly preferred for captive culture, displaying planktonic nauplii that can be captured by cultured larvae in the water column (Schipp, 2006). They can be selectively sieved, separating nauplii (80–250 μm), copepodites (80–350 μm) and adults (250–600 μm). However, the culture of these species is also more demanding, since it requires the use of phytoplankton and low adult densities (Støttrup, 2000). Several authors have already described detailed methodologies for hatchery culture of copepods (e.g. Schipp *et al*., 1999; Payne & Rippingale, 2001; Cutts, 2003). Copepod genera providing the most promising results concerning their production in intensive systems are *Acartia* Dana, 1846, *Calanus* Leach, 1819, *Centropages* Krøyer, 1849, *Paracalanus* Boeck, 1865, *Parvocalanus* Andronov, 1970, *Pseudocalanus* Boeck, 1872 and *Temora* Baird, 1850 (Mauchline, 1998).

A pertinent question is recurrently asked concerning the use of cultured copepods, namely, is the nutritional value of wild copepods retained in cultured specimens? The nutritional value of cultured copepods is known to be largely influenced by their diets (Støttrup *et al*., 1999; Payne & Rippingale, 2000; Lacoste *et al*., 2001), with algal quality directly affecting copepod quality. An adequate mixed diet of several high quality algal species is known to produce better results than a monospecific diet. While *Artemia* boosted by microalgae displays high HUFA levels in storage lipids (triacylglycerols) (Nanton & Castell, 1999), cultured copepods exhibit higher HUFA content in structural or polar lipids (Bell *et al*., 2003). In practical terms, HUFA stored in the polar fraction of dietary prey, as displayed by copepods, are more bio-available to cultured larvae (McKinnon *et al*., 2003).

Although Kraul (1999) emphasized the importance of the use of copepods in the culture of marine ornamental shrimps, namely *Hymenocera picta*, no study has ever addressed in detail the suitability of copepods as larval prey for larval ornamental shrimps. None the less, it is possible that these live prey may play a decisive role in the minimization of early larval stage mortality, probably correcting potential nutritional imbalances resulting from the use of 'traditional' larval foods, namely rotifers and *Artemia*. Employing a mesocosm method can be an excellent approach for marine ornamental shrimp culture. Intensive culture methodologies require a high level of biological knowledge and know-how, which unfortunately are still not fully available for these organisms. In these situations, selecting a semi-intensive rearing method such as mesocosm may be a viable option to solve larval rearing bottlenecks (Papandroulakis *et al*., 2004). In mesocosm, rearing conditions are both natural and artificial, with two different approaches being possible according to the origin of the food chain. The first is characterized by an extensive approach,

since the food chain is basically endogenous; it is complemented by exogenous input to keep the density of desirable larval prey stable. The second approach features a more intensive methodology, since the food available for developing larvae is basically exogenous; however, food items present the ability to reproduce endogenously owing to both the low density of larvae usually employed in this culture system and the presence of phytoplankton (Papandroulakis *et al*., 2004). Using a high quality mix of microalgae, rotifers, *Artemia* and copepods as potential food sources in a mesocosm allows cultured larvae to fulfil their energetic requirements until metamorphosis. Basically, larvae can select the most suitable prey(s) during each zoeal stage, taking advantage of the diversity of sizes displayed by the different life stages of copepods and *Artemia*. Newly hatched larvae will have available microalgae, rotifers, *Artemia* nauplii and copepods (including nauplii, copepodites and adults), whereas late zoeal stages will be able to fulfil their higher energetic requirements by preying on juvenile and/or adult *Artemia*. Obviously, the nutritional suitability of each prey will always depend on the quality of the microalgae being provided to the mesocosm. Additionally, special attention must be paid to ensure that a photoperiod suitable for microalgae is employed, as well as for selected copepod species since it can significantly affect the egg production of copepods (Peck & Holste, 2006).

None the less, only after experimentally proving that the economic costs of keeping copepod cultures and mesocosms results in higher revenues for enterprises, expressed in significantly higher survival rates to metamorphosis, will this approach be a viable option for marine ornamental species culture.

7.3.5 Other dietary items

Because of the large size attained by late zoeal stages of caridean and stenopodidean shrimps, researchers have quickly realized that larval foods commonly employed to culture decapods in captivity may have a limited application. The ability of larval decapods to capture large-sized prey items was early recorded by Lebour (1925), describing the ability of spiny lobster phyllosoma larvae to capture young fishes. Gurney & Lebour (1941) and Gurney (1942) reported how they were able to keep late zoeal stages of caridean shrimps in captivity until metamorphosis by feeding them small pieces of chopped mussel and fish. Curiously, after the generalization of the use of newly hatched *Artemia* nauplii to raise larval decapods, this practice was mostly forgotten, only being occasionally used by researchers raising decapods to describe their larval morphology. It was only after the successful use of mussel gonad to feed late-stage spiny lobster phyllosoma larvae (Kittaka, 1994a, b) that such dietary items were once again considered as a potential option for larval decapod culture.

Concerning the culture of marine ornamental shrimps, Palmtag & Holt (2001) described the successful use of a frozen mix composed of 50% puréed squid

and 50% puréed shrimp to feed late zoeal stages of the fire shrimp *L. debelius*. The authors administered this diet by thawing small portions of the mixture every morning and placing them on the water surface of the larval culture vessel. In a later work, Palmtag & Holt (2007) gave cultured *L. debelius* larvae a strip of thawed, beheaded, peeled shrimp cut lengthwise (approximately 0.5 g each). The authors used a specially designed feeding ring to supply the shrimp piece to cultured larvae. This device was composed of a ring formed by a cable tie connected to itself, with a small hole drilled into it. This hole allowed one end of a toothpick to be inserted in the cable tie and the other end into the strip of shrimp. The ring was suspended over the rearing chamber, with the shrimp strip being placed below the water surface. Grating frozen squid and supplying the small shredded pieces to cultured larvae has also proved to be highly effective to supplement an enriched *Artemia* metanauplii diet used to culture late-stage *L. amboinensis*, *L. debelius* and *L. seticaudata* (Calado, unpubl. obs.). Despite the promising results achieved, Palmtag & Holt (2007) noted that larval *L. debelius* seemed to prefer smaller food particles, although cultured larvae could extract tissue from the suspended shrimp strip. Apparently small food particles may improve cultured larval ability to manipulate their food and increase their intake. Additionally, smaller particles are more easily spread throughout the rearing tank, which can significantly increase encounter rates between these particles and cultured larvae. None the less, Barros & Valenti (2003) did not record any preference for particle size by *Macrobrachium rosenbergii* during larval development. Certain biochemical compounds (e.g. amino acids, sugars and lactic acid) are known to stimulate feeding responses in crustaceans (Carr *et al*., 1984; Coman *et al*., 1996). Palmtag & Holt (2007) suggested that puréed shrimp may have initiated and/or accelerated the release of chemoattractants through their higher surface area exposure when compared with suspended shrimp strips. If so, cultured larvae fed puréed shrimp or grated squid may display higher feeding rates, since they may be able to detect food particles more efficiently.

A major drawback resulting from the use of these dietary food items is the sharp decrease in water quality, mainly when using puréed or minced foods. This practice also promotes the development of higher microbiological loads, which increase the chances of cultured larvae being affected by pathogens. Effective biological filtration systems, coupled with the daily siphoning of uneaten food particles, may help to mitigate the negative effects described above. However, regular tank cleaning is a tedious and time-consuming operation, which may not be viable in large commercial-scale culture. Despite the clear benefits described above, cultured larvae fed such dietary items will always be more exposed to higher risks of sudden mass mortality than when using 'traditional' live prey. In conclusion, the receptivity of marine ornamental shrimp larvae to fresh and frozen pieces of various foods (namely squid and shrimp) is an excellent indicator of the potential use of formulated diets during larval culture.

7.3.6 Formulated larval diets and digestibility issues

Formulated diets serve as partial or complete replacements for live food, minimizing production costs and eliminating the variation in the nutritional quality of live foods. During the last two decades significant progress has been achieved in microdiet development, either microbound, microencapsulated, or microcoated (Tucker, 1998). The lower price of formulated diets when compared with the prices of live prey such as *Artemia* nauplii or enriched metanauplii, makes their inclusion in decapod larval diets a cost-effective choice. The success of a given larval diet is highly dependent on its shape, size, moisture content and binder (D'Abramo, 2002). Caridean and stenopodidean larvae use their mouthparts to grasp and manipulate food particles, being able to modify them into smaller portions that may be ingested. In this way, pellet size and shape may not be as critical as for other larval decapods (e.g. the filter feeding early larval stages of penaeid shrimps). However, it is also known that particle size acceptance increases with larval stage, with advanced zoeal stages accepting larger food particles (Jones *et al.*, 1979, 1987; Genodepa *et al.*, 2004a). As stressed by Johnston & Johnston (2007) for spiny lobster larvae, larval ability to modify particle size requires the high physical integrity of formulated diets. Diet stability is usually enhanced by the use of different binders or extrusion methods (Teshima *et al.*, 1982; Teshima & Kanazawa, 1983b; Jussila & Evans, 1998). Diets with high levels of moisture presumably allow a faster release of chemoattractants, which stimulate feeding behavior (Tolomei *et al.*, 2003). D'Abramo (2002) notes that an increased feeding response by larvae to diets with higher moisture content suggests that their levels of palatability and consumption are superior to those of dry diets. It is known that diets bound with the use of binders are commonly more prone to leach water-soluble nutrients (Johnston & Johnston, 2007). Although some leaching is necessary to promote feeding in decapods, excessive nutrient leaching may significantly reduce the nutritional value of diets and contribute to a decrease in water quality (López-Alvarado *et al.*, 1994; Teshima *et al.*, 2006). This trade-off between water stability and minimal leaching is commonly achieved by partly sacrificing diet attraction and even palatability (D'Abramo, 2002).

It has been suggested that the softer texture of diets with high moisture levels may mimic the natural live prey of early-stage phyllosoma larvae of spiny lobsters, which is thought to consist of gelatinous zooplankton (Macmillan *et al.*, 1997; Cox & Johnston, 2003a). Several works have already addressed the morphological and functional variations of mouthparts and foregut through the larval development of spiny lobsters (Johnston & Ritar, 2001; Cox & Johnston, 2003b). In fact, potential natural prey items have even been identified through the analysis of signature lipids (Jeffs *et al.*, 2004). Such studies are still unavailable for marine ornamental shrimp larvae, which makes difficult the formulation of diets with suitable texture and moisture levels. None the less, the preference for soft/moist diets has already been determined for a wide

range of marine and freshwater shrimps (Subrahmanyam & Oppenheimer, 1970; Kitabayashi *et al*., 1971; Kovalenko *et al*., 2002).

Rhyne & Lin (2004) were the first to clearly demonstrate the potential use of inert diets to culture the larval stages of marine ornamental shrimps. As reported for many other shrimp larvae (e.g. Kumlu & Jones, 1995; Brito *et al*., 2001; Robinson *et al*., 2005), Rhyne & Lin (2004) were able to partially replace live prey (*Artemia* nauplii and enriched metanauplii) by an inert diet (ArteMac^{TM}) during the larval culture of peppermint shrimps *Lysmata*. The first evidence suggesting that marine ornamental shrimps could accept inert diets was recorded by Rhyne *et al.* (2001) through the use of high-speed video analysis. In fact, D'Abramo (2002) suggests that a correct evaluation of the acceptance of formulated diets may only be properly ascertained through a detailed video analysis of the feeding behavior of cultured larvae. The main reason for this approach is that a diet may have a suitable size and texture, as well as an adequate nutrient profile, but still not be accepted by cultured larvae.

Rhyne *et al*. (2001) verified how the presence of live prey (*Artemia* nauplii) stimulated *Lysmata* larvae to capture and ingest a higher number of inert diet particles. Co-feeding live feeds with formulated diets has also been highly successful with other larval decapods (e.g. Biedenbach *et al*., 1990; Genodepa *et al*., 2004b; Fiore & Tlusty, 2005; Johnston *et al*., 2008), whereas feeding newly hatched larvae exclusively with inert diets commonly results in lower survival (Johnston *et al*., 2005). *Artemia* appears to release chemical cues that increase the acceptance of formulated diets. Additionally, the biochemical composition of live prey may improve the digestibility and assimilation of formulated diets. Larval enzyme activities, as well as digestive capacities, have always been regarded as the likeliest explanations for the success (or failure) of formulated diets (D'Abramo, 2002). Researchers have speculated that larval digestive capacity is lower than that required to digest formulated diets effectively and that exogenous enzymes supplied by *Artemia* nauplii could play a vital role (Lovett & Felder, 1990a, b). Curiously, Jones *et al.* (1993) and Kamarudin *et al.* (1994) have shown that the contribution of exogenous enzyme from *Artemia* to the digestion process of larval shrimps appears to be insignificant. It has been suggested that, under conditions of high food availability similar to those present in laboratory larval cultures, digestive enzyme levels may be reduced, since energy requirements are fulfilled without the need for highly efficient digestion (Harms *et al*., 1991). In the same way, at lower food levels, excluding situations of starvation or malnutrition, higher digestive enzyme content can be expected (Harris *et al*., 1986). Comparative studies on digestive enzymes, levels of activity and changes during ontogeny are crucial to fully understand the nutritional needs of cultured larvae through their entire larval cycle. Concerning marine ornamental shrimps, only the trypsin levels of the first two zoeal stages of *L. debelius* have been quantified so far (Le Vay *et al*., 2001). Jones *et al.* (1997a) suggested that trypsin content in crustacean larvae can reflect their

adaptations to feeding at a particular trophic level. *Lysmata debelius* larvae display some of the lowest trypsin contents recorded in comparative studies between larval decapods, with similar levels to those displayed by the carnivorous larvae of clawed lobsters (*Homarus* Weber, 1795 and *Nephrops* Leach, 1814) (Le Vay *et al*., 2001). Apparently, these results confirm the assumptions of Fletcher *et al*. (1995) that *L. debelius* larvae are probably obligate carnivores. Future studies in this area may help researchers to formulate more suitable diets for larval marine ornamental shrimps.

Food particle motility through the gut decreases during ontogeny whereas the average retention time increases (Kurmaly *et al*., 1990). Understanding the development of the digestive tract and gland during larval development helps to diagnose at which zoeal stage larvae may display higher digestive capacities and therefore be able to digest formulated diets more readily. This approach has already brought promising results in the culture of larval penaeid shrimps and spiny lobsters (e.g. Lovett & Felder, 1989; Johnston *et al*., 2008). In marine ornamental shrimps the occurrence of mass larval mortalities, or mark-time moulting, at particular zoeal stages (Calado *et al*., 2001a) can be good indicators of larval physiological changes and/or higher energetic requirements.

It is known that a formulated feed is only 'as good as its ingredients' (Glencross *et al*., 2007). Apart from the issues concerning lipid, vitamin and mineral composition of formulated feeds, five out of ten essential amino acids (EAAs) are commonly deficient in formulated diets for crustaceans: lysine, methionine, tryptophan, isoleucine and threonine (Houser & Akiyama, 1997). If the requirements for these amino acids are met, the other five are usually present at sufficient levels to satisfy dietary needs.

In conclusion, balancing the nutrients of formulated diets and improving their acceptance and digestibility by crustacean larvae remain central tasks for researchers. The variability of marine ornamental species currently being traded may lead researchers to focus their research on the most highly priced ones, namely in the genus *Lysmata* and *Stenopus*. None the less, it is possible that nutritional species-specific requirements exist in the same genus, mainly because of the different habitats occupied by these species. Until formulated diets become more efficient, live dietary items will remain the most important food source for the larval feeding of marine ornamental shrimps.

Chapter 8

Grow-out

8.1 Grow-out systems and culture procedures

The constraints still impairing commercial-scale culture of marine ornamental shrimps mean that current information available on juvenile grow-out is limited to a small number of species. So far, the following species have already been cultured in captivity on a commercial scale: *Lysmata amboinensis* (P. West, pers. comm.) (see color plate 8), *L. debelius* (Fosså & Nilsen, 2000; Palmtag & Holt, 2001), *L. seticaudata* (Calado *et al*., 2005e), *Lysmata* spp. from the peppermint complex (A.L. Rhyne, pers. comm.) and *Hymenocera picta* (Kraul, 1999; Fosså & Nilsen, 2000). However, the current protocols for the culture of larval *L. debelius* and *L. amboinensis* cannot yet be relied upon to ensure regular production of these highly priced marine ornamental shrimps. The unpredictable survival of these species to metamorphosis is a serious constraint to any commercial enterprise needing to ensure the supply of a regular number of specimens to customers. Nevertheless, an enterprise culturing *L. debelius* and *L. amboinensis*, even in small amounts and on an irregular basis, may still achieve a higher market value for these captive-bred specimens when supplying conscientious customers willing to avoid marine ornamentals collected from coral reefs. *Lysmata* spp. from the peppermint complex are not primary choices for captive culture in the USA since these shrimps are collected by stone crab and lobster fishermen as well as by bait shrimp trawlers, being an abundant and rather inexpensive shrimp in the aquarium market (Rhyne & Lin, 2004). However, in Europe this species is not commonly available and therefore commands higher market values. The high demand for peppermint shrimps in Europe, particularly to control plagues of glass anemones *Aiptasia* spp. in reef displays (Rhyne *et al*., 2004), has made them much more appealing for captive culture than in the USA. Nevertheless, after Calado *et al*. (2001b) highlighted the potential use of the warm temperate shrimp *L. seticaudata* to control *Aiptasia* in reef aquariums and described a feasible methodology for their larval culture on a commercial scale (Calado *et al*., 2003a), European enterprises shifted their initial interest in the culture of western Atlantic peppermints to autochthonous Monaco shrimps. The culture of juvenile harlequin shrimps *Hymenocera* still faces a

major constraint for their commercial success: after metamorphosis, these organisms stop accepting 'typical' larval diets (e.g. rotifers, *Artemia*, copepods) and shift their feeding habits to a diet strictly composed of starfish (Kraul, 1999). Starfishes in the genus *Linckia* seem to be the favourite food of juvenile *Hymenocera*, although they also accept other starfishes such as the destructive crown-of-thorns *Acanthaster planci*. These shrimps also accept 'alien' starfishes in captivity, namely the abundant eastern Atlantic species *Asterina gibbosa* and *Marthasterias glacialis*. As recognized by Kraul (1999), the marketability of harlequin shrimps will only be improved when an artificial diet is able to successfully replace their starfish dietary requirements. Such an achievement would not only eliminate the need to harvest high numbers of starfish regularly during harlequin shrimp grow-out, but would also increase the popularity of this amazing shrimp among hobbyists, who would be able to keep them much more easily in captivity.

For these reasons the only species currently being cultured on a commercial scale and on a regular basis is the Monaco shrimp, *L. seticaudata*. The main advantages of this species over other peppermint shrimps in the genus *Lysmata* are:

(1) the lower number of larval stages (nine zoeae plus megalopa) and consequently a shorter larval cycle until metamorphosis (under optimum conditions, settlement occurs on the nineteenth day post-hatching) (Calado *et al*., 2004);
(2) the well-established larval culture protocols and the possibility of raising them from hatching until metamorphosis only on newly hatched *Artemia* nauplii (Calado *et al*., 2005a); and
(3) the fact that the ability of cultured specimens to control *Aiptasia* in reef aquariums has already been experimentally demonstrated (Calado & Narciso, 2005).

Whereas penaeids and giant freshwater shrimps are commonly raised in large exterior earth ponds (e.g. Fast & Menasveta, 2000), juvenile marine ornamental shrimps are raised indoors, using smaller-sized containers in recirculated systems. White, black and grey tanks, either opaque or translucent, as well as glass aquariums, have already been successfully employed to raise juvenile marine ornamental shrimps. However, no study has ever addressed the influence of tank coloration on their growth. Although rectangular tanks (varying in volume from 60 to 600 liters) are commonly employed for the grow-out of juvenile *Lysmata*, circular tanks may be a more functional option. At least for fish grow-out, circular tanks present the following advantages (Timmons *et al*., 1998):

(1) they are more simple to maintain;
(2) they provide uniform water quality;

(3) they allow operation over a wide range of rotational velocities to optimize the condition of cultured specimens;
(4) solids (such as uneaten food particles) rapidly settle and are flushed through a central drain; and
(5) they allow the development of systems for visual or automatic observation of waste feed, enabling producers to control satiation and prevent overfeeding.

Indeed, regular tank siphoning is one of the most tedious and time-consuming routines required during the grow-out of juvenile marine ornamental shrimps in rectangular containers. Overlooking this basic hygiene aspect can seriously decrease water quality in indoor recirculated systems. The first warning signal commonly exhibited by marine ornamental shrimps in systems displaying poor water quality is the presence of small, dark spots in their branchial chamber. If water quality is not corrected, these spots grow larger in size and indicate that branchial clogging by suspended material is now seriously affecting cultured shrimps. In extreme situations, opportunistic pathogens may infect the most affected shrimps and induce mortality. However, siphoning grow-out tanks stocked with newly settled shrimps while not siphoning the shrimps themselves is a challenging task. The accidental siphoning of newly settled shrimps commonly results in some sort of physical damage, usually the autotomy of pereiopods or the loss of antennal flagella. These injuries make damaged shrimps more vulnerable to the action of opportunistic pathogens. To avoid these situations, it is advisable to employ a small screen in the tip of the siphoning device with a mesh size that allows solid particles to be properly removed from the culture tanks while preventing newly settled shrimps from being siphoned.

Since ornamental shrimps do not swim in the water column, a suitable tank for grow-out of juveniles should present a high surface area/volume ratio. Employing shallow tanks has the following advantages:

(1) their use reduces the water volume of recirculated systems, which may decrease the financial investment in life support equipment and marine salt mix when employing synthetic sea water;
(2) they allow the condition of cultured specimens to be monitored more effectively; and
(3) their use facilitates routine cleaning tasks.

However, such containers have negative aspects:

(1) their larger air/water interface may promote higher evaporation, which will be reflected in water salinity fluctuations and osmotic stress;
(2) because of their larger water surface, a higher energetic expense may be required to heat or cool the water; and

(3) if the water level is too close to the top of the tank walls, cultured shrimps may easily jump out of the tank when distressed.

The surface area of culture tanks may be easily increased by placing objects with high surface area/volume ratios inside them, which can significantly improve shrimp production (Tidwell *et al.*, 1999, 2000, 2002). Apart from increasing available surface area, these objects improve biological filtration, act as shelters for cultured shrimps and may even act as an additional source of food for cultured shrimps as a result of the development of biofilms (Bratvold & Browdy, 2001; Ballester *et al.*, 2007). In marine ornamental shrimp culture, the objects most commonly employed for these purposes are small pieces of live rock, mesh bags, artificial grass mats and stacked polyvinyl chloride (PVC) pipes. The use of live rock pieces may make it difficult to carry out routine tank cleaning and shrimp observation, and may promote the accumulation of detritus beneath the pieces and disturb water flow dynamics. Additionally, unwanted pest organisms (e.g. fire worms *Hermodice* spp. and glass anemones *Aiptasia* spp.) may be involuntarily introduced in culture tanks through the addition of live rock (Calado *et al.*, 2007c). The delicate texture of early grow-out phases means that the use of mesh bags and artificial grass mats is likely to be more appropriate when newly settled shrimps are stocked. These structures provide shade and encourage shrimp to forage. The cryptic nature of most marine ornamental shrimps commonly prevents them from venturing outside their shelters to seek for food particles if the culture vessel is brightly illuminated. A high light intensity is not, therefore, recommended during the grow-out of marine ornamental shrimps, since their strongly positive phototactic behavior, displayed during early larval development, is reversed to a negative phototactic response after settlement (Calado, unpubl. obs.). The small shelters provided by these structures may be vital to protect smaller shrimps from being cannibalized by larger conspecifics, particularly during the moult period. Again, the mesh bags and artificial grass mats must be in such a position as to ensure that there is minimal interference with the water flow dynamics inside the culture tank and that no 'dead spots' exist. Such 'dead spots' can be easily recognized in a culture tank if detritus accumulation is continuously recorded in the same region of the tank.

Polyvinyl chloride (PVC) pipes (60 mm long and 25 mm in diameter) are commonly glued in stacks of 5 rows by 10 columns, provided for shelter at a proportion of 1 shelter per 50 shrimps (Figure 8.1) (Calado & Dinis, 2007). The small space available between stacked pipes is ideal for smaller shrimps to seek shelter, avoiding larger conspecifics during ecdysis. These structures can be easily cleaned and should be positioned parallel to predominant water flow, allowing food particles to enter the pipes to be captured by shrimps seeking shelter inside. The use of these structures is very practical when collecting cultured shrimp for periodic survival monitoring and size grading, since shrimps quickly seek shelter inside the PVC pipes when disturbed. This

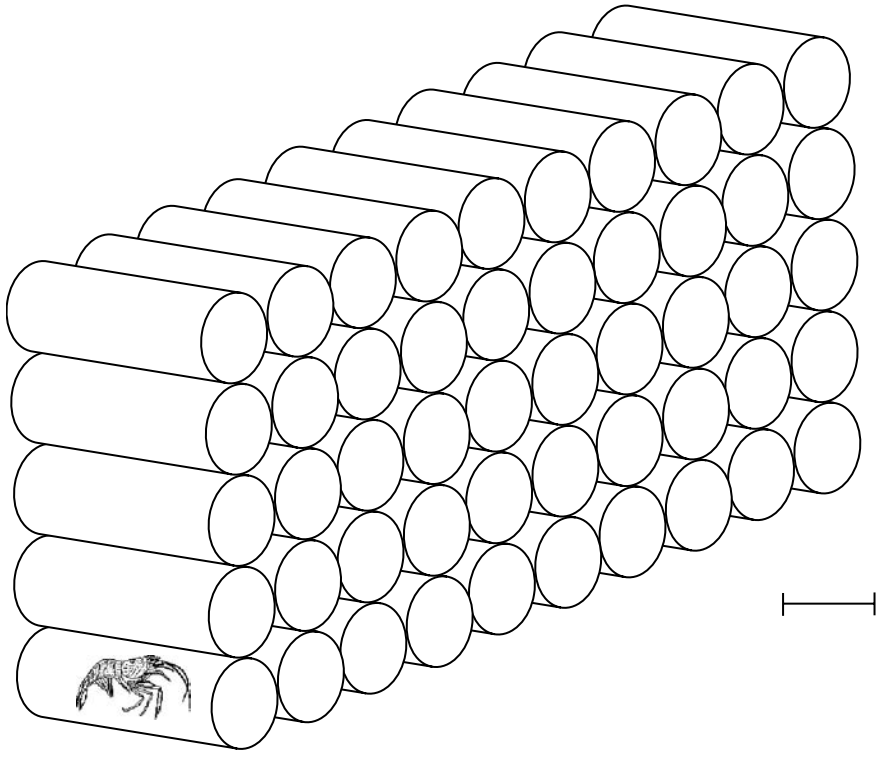


Figure 8.1 Shrimp shelters built of PVC pipes (25 mm diameter and 60 mm long) glued in stacks of five rows by ten columns, provided during the grow-out period at a proportion of one shelter for every 50 newly metamorphosed shrimps (scale bar 20 mm).

behavior allows the structure to be gently removed from the bottom of the tank and rapidly emptied into a container to collect the majority of the cultured shrimps.

There is still a great lack of knowledge concerning which are the most suitable stocking densities during the grow-out phase of marine ornamental shrimps. In fact, some species may not even be suitable for communal rearing. The most evident examples are the highly territorial species of boxing shrimps in the genus *Stenopus*. Other marine ornamental shrimps occurring in the wild as mated pairs, such as pair-living *Lysmata* (*L. debelius* and *L. grabhami*), may also be unsuitable for long-term communal rearing. For species displaying high intraspecific levels of aggression it may be necessary to employ individual grow-out techniques popularized by the aquaculture of clawed lobsters (e.g. *Homarus* spp.) or crayfish (e.g. *Cherax* spp.) (Manor *et al.*, 2002; Kristiansen *et al.*, 2004). Chelae immobilization during grow-out, as described for clawed lobsters (Kendall *et al.*, 1982), is not an option for marine ornamental shrimps. Apart from being a tedious and time-consuming operation, new chelae immobilization would be required after each moult. Given that

caridean and stenopodidean shrimps display short intermoult intervals during their juvenile phase, it is clear that the amount of work required to make sure that all shrimps have their chelae fastened during the entire grow-out period is unrealistic. Additionally, these organisms are not as robust as clawed lobsters and could easily be damaged during this process. The ablation of the chelae's dactylus as suggested by Kendall *et al.* (1982) would greatly decrease the marketability of marine ornamental shrimps and is certainly not a viable solution.

Trays with individual compartments have been successfully used to raise juvenile clawed lobsters. These units are made of plastic, with a perforated bottom, and may be dimensioned to fit cultured specimens until a desired body size. Kristiansen *et al.* (2004) reported that a 60 mm × 130 mm compartment is suitable for raising a clawed lobster until it reaches 80 mm in total length. These units were originally designed for circulating in circular channels, with individual compartments being fed when passing under a feeding bridge (Grimsen *et al.*, 1987). The negative aspects of this system are poor water circulation, which promotes rapid degradation of water quality under intensive culture, and the large area required to implement these units.

Car-o-cells/horizontal wheels are an interesting option for culturing decapods individually. A wheel is divided into concentric circles, which are again divided into individual compartments of variable sizes that are positioned around a central area to which inflowing water is supplied. Water overpressure in the central area ensures an even distribution of water flow through the perforated walls of individual compartments (Kristiansen *et al.*, 2004). The wheel can easily be turned, allowing each compartment to be inspected from a single position at the tank wall. A compromise must be achieved between the diameter of the holes drilled in the compartment walls and floor, in order to allow adequate water flow while preventing cultured specimens from attacking conspecifics in adjacent compartments. It is also important that the holes drilled on the side walls of the compartment are not too close to the top, creating a slippery area that would prevent cultured specimens from climbing the walls and entering adjacent compartments.

The stacks system described by Kristiansen *et al.* (2004) displays five to seven trays vertically stacked in 1 m^2 square tanks with 70 cm of water depth. Each tray could hold up to 180 cultured specimens divided by individual compartments (size 36 cm^2). The water flow through the stacks is achieved by an overpressure in the top tray, ensuring an equal water flow into each compartment. This system displays two significant drawbacks:

(1) during feeding and inspection, each tray must be removed from the stack and taken out of the water; this can be a major stress factor for cultured shrimps, and particularly for newly moulted specimens; and
(2) it does not display an adequate self-cleaning mechanism, leading to detritus accumulation inside the square tank holding the trays.

In any three-dimensional system for individual culture that tries to maximize available grow-out area by using the whole water volume, providing an adequate water flow is far from being a simple task. To overcome water quality problems, individual compartments are perforated and allow chemical contact (through waterborne substances), visual contact and even limited tactile contact between neighbouring specimens. An interesting experiment by Barki *et al.* (2006) using juvenile redclaw crayfish *Cherax quadricarinatus* (Von Martens, 1868) in a system of three-dimensional individual compartments, revealed the influence of neighbouring conspecifics on their growth performance. Stocked specimens revealed significantly lower growth rates when surrounded by neighbours on all sides, in opposition to those reared with no neighbours in adjacent compartments, or above or below them. Barki *et al.* (2006) also revealed how the growth rate of smaller specimens surrounded by larger conspecifics was negatively affected. This study highlights the important role played by social factors in decapod grow-out, not only for species forming stable mated pairs in the wild, but also for those occurring in large aggregations.

When culturing ornamental shrimps apparently suitable for communal rearing (e.g. those known to occur in groups of several individuals in the wild), agonistic intraspecific interactions may still be a problematic issue. Obviously, these problems are amplified when high stocking densities are used during grow-out. Calado & Dinis (2007) noticed such negative aspects when they increased the culture density for juvenile Monaco shrimps *L. seticaudata*, a gregarious shrimp species. The most evident cause of mortality during grow-out was cannibalism, even when food items were available. Cannibalization of conspecifics is more pronounced during the moult, when even small shrimps may attack larger sized individuals. The addition of live *Artemia* nauplii, metanauplii or adults to grow-out tanks has already proven to be a potential solution to the need to minimize cannibalism. The presence of live prey stimulates the predatory behavior of cultured shrimps, which tend to ignore even newly moulted conspecifics. Although no quantitative studies have been performed, it seems that the presence of live *Artemia* also stimulates the ingestion of grow-out diets. When using live *Artemia* nauplii or metanauplii on grow-out tanks, it is necessary to employ a screen with suitable mesh size on the tank's outflow in order to prevent these prey from being rapidly drained. Additionally, these screens must be regularly cleaned since high water inflow may rapidly clog them.

Stocking grow-out tanks with similarly sized newly settled shrimps is vital to minimize the occurrence of heterogeneous individual growth and maximize culture profitability (Ranjeet & Kurup, 2002; New, 2005). Heterogeneous individual growth can rapidly become a serious bottleneck to commercial-scale culture, since larger individuals will become even larger during grow-out, will easily outcompete smaller conspecifics for food, and will significantly increase the risk of larger shrimps cannibalizing smaller ones. Smaller sized individuals

deprived of adequate food levels during the initial periods of grow-out will take a much longer time to reach a marketable size, even when they are placed with similar sized conspecifics at lower densities and provided with suitable dietary regimes (Calado, unpubl. obs.).

The following factors may be regarded as potential inducers of heterogeneous individual growth:

(1) Intrinsic factors, such as genetic differences and age at metamorphosis (Sandifer & Smith, 1979); Calado *et al.* (2005a) recorded the occurrence of highly variable size in newly metamorphosed *L. seticaudata* originating from larvae cultured under suboptimal conditions that promoted asynchronous settlement.
(2) Intraspecific competition, namely when space and food are limiting factors.
(3) Social aspects, particularly the position within size hierarchy and territoriality; this factor is of major importance in marine ornamental species displaying complex social structures (e.g. *Rhynchocinetes*) (Correa *et al.*, 2000, 2003) and those whose sexual differentiation can be influenced by social environment (e.g. *Lysmata*) (Lin & Zhang, 2001a; Baeza & Bauer, 2004; Baeza, 2007a).

In order to minimize the existence of heterogeneous individual growth during juvenile ornamental shrimp culture, the following procedures should be addressed:

(1) If possible, always stock shrimps in grow-out tanks which have settled on the same day, or which do not have an age difference greater than 5 days (considering the day of metamorphosis as day 0).
(2) If not enough shrimps are available to stock a grow-out tank with an age difference less than 5 days, only stock newly settled shrimps with a maximum age difference of 10 to 15 days; shrimps with an age difference of more than 10 days have already moulted at least twice and have a considerable feeding advantage over newly settled shrimps.
(3) If only a reduced number of newly settled shrimps are produced, individual culture (even of species which allow communal rearing) should be preferred; although it is more labour intensive, it also promotes higher growth rates and maximizes survival (see Calado & Dinis, 2007).
(4) Shrimps must be sorted by size at least 3 to 4 weeks (or when heterogeneous individual growth becomes evident) after being stocked in grow-out tanks; they should be distributed in commercial size classes (e.g. the classes proposed by Calado & Dinis (2007): not commercial, total length < 20 mm; tiny, 20 mm $<$ total length < 30 mm; and small, 30 mm $<$ total length $<$ 40 mm); specimens from different batches but in the same commercial size class should be pooled together and placed in a different grow-out tank.

(5) Adequately decrease rearing density with increasing commercial size class.

Although it has never been tested in captivity, at least not on a commercial scale, the grow-out of alpheid shrimps may require specially designed tanks. This assumption is based on the potential need to add substrate to the bottom of the culture tank, because of the shrimps' burrowing behavior. The study by Palomar *et al.* (2004) on *Alpheus macellarius* (Chace, 1988) revealed that this species is a sediment scavenger and that it displays a predominant deposit-feeding mode. These observations were supported by the shrimp's behavior and burrow architecture. Additionally, Palomar *et al.* (2005) also verified in laboratory trials that shrimps stocked with 15% and 25% gravel substrates produced more burrows than shrimps stocked with 0% and 5% gravel substrates. The authors also noted that the use of 15% and 25% gravel substrates favoured a higher stability and a more complex architecture of the shrimp burrows. The presence of substrate in the bottom of the tank makes routine cleaning difficult, and the area at the bottom of the tank may need to be large in order to ensure that shrimps do not display agonistic behaviors as a result of the proximity of the burrows of conspecifics.

A curious aspect recorded during the culture of juvenile *L. seticaudata* is the occurrence of precocious sexual phase change (see color plate 10) (Calado *et al.*, 2005e). So far, all species in the genus *Lysmata* are believed to be born as males and later in their life may change to simultaneous hermaphrodites able to produce both male and female gametes (Bauer, 2006). Under intensive culture, employing high water temperatures and stocking densities, juvenile shrimps were observed carrying embryos at much smaller sizes than those recorded for specimens in wild populations (Calado *et al.*, 2005e; Calado & Dinis, 2007). From a commercial viewpoint, the occurrence of precocious sexual phase change is highly disadvantageous, since the shift in the energetic budget from growth towards the production of large and highly energetic vitellogenic oocytes results in a lower growth rate. Although no reliable laboratory data currently exist on the onset of sexual maturation of gonochoric marine ornamental shrimps cultured at high stocking densities, it is possible that female shrimps may also mature earlier than in the wild. An interesting approach that would certainly improve profitability would be to manipulate the sex ratio of cultured specimens, targeting 100% male shrimp production. Monosex cultures of decapod crustaceans have already been addressed for species produced for human consumption (e.g. *Macrobrachium* Bate, 1868, and *Cherax* Erichson, 1846), preventing energy being diverted from growth to reproduction (Curtis & Jones, 1995; Sagi *et al.*, 1997a). Sex determination in shrimps can be affected by diet manipulation during specific periods of their early development (Zupo, 2000, 2001). The apoptotic disruption of the male gonad, as well as the androgenic gland, selectively triggered by the ingestion of benthic diatoms during the fifth to the twelfth

day post-settlement of *Hippolyte inermis* Leach, 1815, was a remarkable finding described by Zupo & Messina (2007). In crustaceans the androgenic gland is the sole source of hormones responsible for sex differentiation (Sagi *et al*., 1997b; Sagi & Khalaila, 2001; Abdu *et al*., 2002). In male crustaceans, the endocrine and gametogenic functions are clearly separated into two distinct organs, the androgenic gland and the testes, respectively (Payen, 1983; Charniaux-Cotton & Payen, 1988). In fact, sex differentiation has already been manipulated through the removal of the androgenic gland in the marine ornamental shrimp *L. seticaudata* (Charniaux-Cotton, 1959a, b, 1961). Ferezou *et al.* (1978) characterized a non-species-specific substance from the androgenic gland named farnesylacetone. If injected in a sexually active female, this lipidic substance inhibits vitellogenesis (Berreur-Bonnenfant *et al*., 1973). According to Berreur-Bonnenfant & Lawrence (1984), farnesylacetone acts by influencing protein and RNA synthesis in its target organs. Several studies have also pointed towards the existence of a proteinaceous androgenic hormone in decapod crustaceans (Sagi & Aflalo, 2005). Recently, Manor *et al.* (2007) reported the case of Cq-IAG, an insulin/insulin-like growth factor family member expressed in a gender-specific manner, exclusively in male crayfish. The authors suggest that the discovery of this gender-specific insulin-like family member may indicate that insulins may have evolved in the context of regulating sexual differentiation. As stressed by Sagi & Aflalo (2005), the development of biotechnology to control sex determination, through the manipulation of androgenic gland activity in decapods displaying bimodal growth, will play a major role in the development of monosex cultures that can maximize production.

Another option to impair maturation of cultured shrimps, even if these change from male to female or simultaneous hermaphrodites, will be to manipulate the biochemical profiles of grow-out diets. It is known that certain nutrients play a key role in the ovarian maturation of decapod crustaceans in captivity (e.g. cholesterol and essential fatty acids) (Harrison, 1990). If formulated diets exclude nutrients essential for ovarian maturation while still promoting good survival and growth rates, precocious sex phase change may not be impaired but at least shrimps will not 'waste' energy producing oocytes.

For simultaneous hermaphrodites, precocious sexual phase change appears to be avoided for longer periods if shrimps are cultured in individual compartments (Calado & Dinis, 2007). Future studies clarifying the physiological processes involved in the extension of male phase duration and sex-specific factors recorded for *Lysmata* (Baeza, 2007b, c) are vital for the development of 100% male shrimp culture protocols. In the same way, studies clarifying how sexual maturity is promoted and/or suppressed in females of gonochoric species may prove to be important to maximize the growth performance of juvenile marine ornamental shrimps.

Biosecurity is a major concern in shrimp facilities worldwide, due to the outbursts of several diseases that threaten shrimp production. The most

devastating ones are viral diseases, namely yellow-head virus and the white spot syndrome virus (Menasveta, 2002).

Although it is not likely, in extreme situations facilities that culture marine ornamental shrimps may either face similar problems and/or act as potential vectors for the dispersal of pathogens (e.g. water discharges to nearby coastal areas). Facilities culturing marine ornamental shrimps commonly employ recirculated systems and are located inland. Therefore, even if an enterprise is affected by a particular disease, it is not likely that it will spread to adjacent facilities, particularly if the company is equipped with disease prevention systems. Apart from regular hygiene procedures employed in any intensive aquaculture facility, some other measures include:

(1) screening and disinfecting influent water, or simply using synthetic sea water;
(2) reducing water exchange rates in facilities using natural sea water;
(3) reducing the microbiological and organic load of effluent water; and
(4) stocking disease-free broodstock and juveniles.

The smaller size of facilities culturing marine ornamental shrimps, when compared with those used to culture penaeid shrimps or *Macrobrachium*, certainly facilitates the practice and enforcement of biosecure procedures. Although major disease problems will probably never affect enterprises culturing marine ornamental shrimps, disease control and environmental friendly culture must always be priorities.

8.2 Grow-out diets, and nutritional and energetic issues

Currently, the majority of studies concerning artificial diet formulation for juvenile shrimp grow-out have focused mainly on species for human consumption. The studies available on the nutritional requirements of juvenile caridean shrimps during grow-out have mostly addressed the nutritional aspects of freshwater palaemonid species in the genus *Macrobrachium*. The different feeding habits and habitats of these species mean that grow-out diets developed for these shrimps will probably not be nutritionally balanced for marine ornamental shrimps.

Despite the almost total lack of knowledge on the nutritional requirements of marine ornamental shrimps during grow-out, the methodologies available to formulate balanced feeds for shrimps cultured for human consumption have a valuable role to play (Tacon, 1996; Hari & Kurup, 2003; Smith *et al*., 2005). Current advances in the development of suitable grow-out diets for juvenile clawed lobsters (Tlusty *et al*., 2005a, b), spiny lobsters (Johnston *et al*., 2003; Smith *et al*., 2003) and crabs (Sheen & Wu, 1999; Catacutan, 2002; Zmora *et al*.,

2005) will also be important for the development of suitable diets for marine ornamental shrimps.

With the exception of harlequin shrimps *Hymenocera*, which feed exclusively on sea stars after metamorphosis, juvenile marine ornamental shrimps are known to accept an incredible variety of foods: decapsulated cysts, live or frozen newly hatched *nauplii*, enriched metanauplii or adult *Artemia,* fresh or frozen chopped squid, shrimp, clams, mussel or fish roe, mixed commercial frozen foods (e.g. Marine Cuisine™ and Emerald Entree™), flakes or freeze-dried Cyclop-eeze™, and shrimp pellets as well as fish flakes and pellets (Calado & Dinis, 2007; Calado *et al*., 2005e, 2007c). The few existing studies have shown the importance of feeding a varied diet, rather than employing single-item dietary regimes (Calado *et al*., 2005e). Although the goal of the study by Calado & Dinis (2007) was not to establish a suitable diet for the grow-out of juvenile *L. seticaudata*, the authors clearly showed how a varied frozen diet (Marine Cuisine™) could promote excellent growth performances significantly superior to those recorded in natural populations. However, the best result ever recorded in communal culture of *L. seticaudata* was achieved with individuals fed live *Artemia* adults. Three hundred newly settled *L. seticaudata* (total length of approximately 8 mm) were divided into three groups of 100 individuals and stocked in rectangular tanks (bottom area 60 cm × 40 cm), at 26°C water temperature and with each tank provided with two stacks of PVC pipes for shelter (described in the previous section). All shrimps were fed with live *Artemia* adults twice a day and allowed to feed *ad libitum*. After 1 month of grow-out, the regular period of time after which the first sorting of cultured shrimps is performed to prevent heterogeneous individual growth, 100% survival was recorded in all tanks, and all cultured shrimps had reached commercial size classes (total length > 30 mm) (Calado, unpubl. obs.). These remarkable results were achieved with adult *Artemia* collected from the wild. The absence of cannibalism during marine ornamental shrimp grow-out is not a common occurrence during intensive culture trials. Apparently, the presence of live prey stimulates the predatory behavior of cultured shrimps and diverts their attention from conspecifics, particularly during the moulting cycle. Another important advantage recorded during this experimental trial was that grow-out tanks had to be cleaned only once a week as a result of the high level of acceptance of this food and through juvenile shrimps completely ingesting their prey. When employing inert diets (fresh, frozen or formulated), grow-out tanks need to be siphoned for food leftovers at least every other day, in order to prevent a decrease in water quality. Unfortunately, live adult *Artemia* is not always available, neither in the wild nor from commercial suppliers. Even when culturing organisms that have high market values and are sold on a unitary basis (not on a weight basis as shrimps for human consumption are), live adult *Artemia* is an expensive food item. Although adult *Artemia* can be successfully cultured (Zmora *et al*., 2002), the financial efforts required may not be acceptable for small-sized enterprises. Another drawback

of the use of live adult *Artemia* is the potential introduction of pathogens in grow-out systems. In this way, live adult *Artemia* should be regarded more as a complementary food and as a potential way to decrease cannibalism during intensive shrimp culture.

It may be legitimate to question the need to develop formulated diets for marine ornamental shrimps given the satisfactory results commonly achieved using a variety of fresh, frozen and dry foods. However, it is unlikely that, without suitable formulated diets, enterprises will be able to undergo commercial-scale culture and produce enough marine ornamental shrimps to fulfil worldwide demand on a regular and economically feasible basis. Additionally, without the use of formulated diets it seems unreasonable to expect that cultured specimens may outcompete less expensive wild-caught shrimps available in the aquarium trade.

Optimal levels of specific nutrients displayed by cultured shrimps at the end of experimental grow-out trials may be established through the analysis of the biochemical profile of wild specimens. It is important to note that these values should be regarded as guidelines and do not have to be achieved at 'any' cost. If a particular diet promotes satisfactory growth and survival, although cultured shrimps exhibit lower levels of certain nutrients when compared with wild specimens, the financial costs and benefits required to fine tune the grow-out diet must be carefully analysed.

Experimental set-ups using the factorial method have already been shown to be reliable in the establishment of dietary protein requirements for decapods (Teshima *et al*., 2001, 2006). This approach may also help to identify optimal lipid and carbohydrate dietary levels. Optimum dietary protein levels for decapod crustaceans range from 23 to 60%, which seems to indicate that protein requirements are species specific (Kanazawa, 1989). The diversity of marine ornamental shrimp species with culture potential may become a bottleneck for the formulation of species-specific diets. In a first approach, the development of a 'wide scope' diet suitable for several species in the same genus (or even family) may be a wiser option. After developing such diets, species-specific nutritional improvements may be addressed in order to optimize the growth performance of ornamental shrimp species with higher market value and/or demand (e.g. *L. debelius*, *L. amboinensis* and *S. hispidus*). It is known that protein is essential for tissue growth and maintenance, playing a key role when the goal is to achieve a commercial size as quickly as possible. However, protein is an expensive component of formulated diets and its catabolic use to meet energetic requirements during grow-out must be avoided (Sedgwick, 1979). An optimal grow-out diet must contain enough non-protein ingredients to act as energy sources, which should preferentially be catabolized for energetic requirements. Lipids and carbohydrates commonly play the 'energetic role', allowing cultured specimens to maximize the use of dietary protein for growth purposes (Bautista, 1986). The bimodal use of dietary lipids and carbohydrates in formulated diets for decapods, in order to impair the catabolism of proteins for

energetic requirements, has been widely studied. Lipids have a limited role in the optimization of protein retention in formulated diets (Ackefors *et al.*, 1992; Kanazawa & Koshio, 1994). For this reason, when formulating artificial diets, carbohydrates are more reliable energy sources to spare the use of proteins (Cruz-Suarez *et al.*, 1994; Rosas *et al.*, 2000).

Despite glucose being the main sugar present in the hemolymph of crustaceans, the use of this monosaccharide in formulated diets must be avoided since it promotes lower growth rates, organisms exhibit poorer protein conversion efficiencies, and it can even increase mortality (Cuzon *et al.*, 2001). According to Radford *et al.* (2005), the suitability of carbohydrates to be included in formulated diets can be rapidly evaluated by measuring the glycemic responses of decapods fed these experimental feeds. This procedure is an inexpensive and much faster alternative to traditional long-term grow-out trials.

When provided with fresh, frozen or even formulated diets commonly used as grow-out diets for juvenile marine ornamental shrimps, cultured specimens display a strong feeding response, but only for the first 30 minutes and usually less (Calado, unpubl. obs.). After this period, shrimps rapidly lose their interest in uneaten food, which remains in the bottom of the culture tank and negatively affects water quality. The inclusion in formulated feeds of ingredients acting as attractants has been proposed as a way to increase consumption rates and consequently growth. When evaluating the action of squid, crustacean and krill meals, fish and krill hydrolysates and a betaine product (Finnstim) as food attractants for formulated feeds for *Penaeus monodon* Fabricius, 1798, Smith *et al.* (2005) verified that cultured shrimps showed a significantly greater preference for feeds containing crustacean or krill meal. Immediately after immersion, food items exhibit a rapid loss of soluble and particulate nitrogenous compounds (Williams *et al.*, 2005). Leaching of amino acids from immersed foods is known to occur very rapidly (Daniel & Bayer, 1987, 1989; Lokkeborg, 1990). Different ingredients and their chemical composition are known to affect the production characteristics of animal feed pellets (Thomas *et al.*, 1998), and the functional properties of ingredients used in feeds formulation are altered during processing due to protein crosslinking (Gerrard, 2002). It is evident that the loss of attractiveness of food items following immersion is caused by the exhaustion of leached soluble compounds. The chemical compounds that promote stronger feeding responses are generally < 1000 Da in molecular weight, soluble in water, non-lipid and amphoteric or basic in charge (Williams *et al.*, 2005). Commonly, these compounds are free amino acids, nucleotides, nucleosides, organic acids or tertiary amine compounds and only rarely lipids. Williams *et al.* (2005) reported that the chemicals that elicit a stronger feed attractant stimulus are the free amino acids taurine and glycine, the nucleotide adenosine 5-triphosphate, the quaternary ammonium compound betaine and combinations of these compounds. Several authors have already shown that mixtures of these

chemicals promote a positive synergetic effect by triggering stronger feeding responses than the ones recorded when these elements are added individually (Mackie, 1973; Daniel & Derby, 1991; Derby, 2000). Artificial diets developed for marine ornamental shrimps must try to minimize the loss of these attractants after immersion, although a limited leaching of those compounds must occur in order to attract cultured specimens towards the feed.

As recorded for other shrimps (Costa-Pierce & Laws, 1985; Pittet *et al.*, 1996), positive growth enhancement may be achieved for marine ornamental shrimps if adequate levels of attractants are added to formulated diets. If cultured specimens are receptive to formulated diets for a longer period of time, the grow-out phase to commercial size may be shortened and significantly decrease food wastage. Improving the acceptance of nutritionally balanced diets will ultimately reduce production costs and increase profitability and marketability of marine ornamental shrimps cultured in captivity.

The inclusion of fish- and poultry-derived ingredients in formulated diets generates substantially greater impacts on effluent water than quality crop-derived ingredients. Whenever possible, diets in general, and those for marine ornamental species in particular, should be formulated in an eco-friendly way. This approach reinforces the sustainability and environmental protection standards that have always been advocated by those involved in the culture of marine ornamentals.

8.3 Wild juvenile shrimps raised in captivity

In order to avoid the current bottlenecks affecting larval culture of marine ornamental species, alternative culture approaches have started to be evaluated. Several coral species, either soft or hard, are now asexually propagated, either *in situ* or *ex situ* (Ellis & Sharron, 1999; Ellis & Ellis, 2002; Calfo, 2007), making use of their remarkable ability to reproduce by fragmentation (Highsmith, 1982). The collection of post-larval marine ornamental fishes from the wild has also been regarded as a suitable alternative for the production of highly priced species (Durville *et al.*, 2003). Crest nets (nets put on the reef crests) (Lecchini *et al.*, 2006), light traps (Doherty, 1987; Dufour & Galzin, 1993), or specially designed collection devices (such as the CARE, 'collect by artificial reef ecofriendly') (Lecaillon, 2004) have successfully been employed to collect large numbers of wild post-larval marine ornamental fishes. The rationale for the harvesting of fish post-larvae, specifically at this development stage, is that these organisms are very abundant before settlement but will suffer high mortalities during the settlement process in coral reefs (Carr & Hixon, 1995; Doherty *et al.*, 2004). If specimens are collected just before settlement, thus avoiding these events of intense predation (Hixon, 1991), the impact of this activity may be negligible (Lecaillon, 2004). Collected specimens are then grown in captivity, being fed with specially formulated artificial diets.

It is important to point out that grow-out operations using very small juveniles, rather than post-larvae, have a significantly higher impact in wild populations, since collection takes place after the intense predation period of settlement in coral reefs. Marine ornamental fishes produced in captivity from post-larvae collected from the wild are less sensitive to shipping, are more easily adapted to life in captivity and readily accept commercial aquarium foods. For these reasons, captive-raised marine ornamental fishes command higher market values than those collected from the wild.

In order to overcome the bottlenecks impeding the culture of marine ornamental shrimps, a similar approach to that described for ornamental fishes has already started to be evaluated. Hair *et al.* (2004) employed crest nets to collect marine ornamental fish post-larvae for grow-out, but rapidly verified that the most valuable and abundant ornamental species being collected were newly settled boxing shrimps from the genus *Stenopus*. According to Hair *et al.* (2004), modified crest nets (with a rigid water retaining cod-end) collected more shrimps, minimized mortality and improved the general condition of harvested shrimps. These collection devices were deployed in the week leading up to and following the new moon, with collected specimens being retrieved early each morning. Collected *Stenopus* specimens were cultured in submerged cages filled with live rock and placed on trestles, in order to keep them off the sea floor. Cultured shrimps were fed twice a day with a diet of minced fish, crustaceans and molluscs. Even when reducing stocking density, early communal rearing trials using submerged cages promoted high mortality rates (between 40 and 88%). These poor results were mainly due to the agonistic interactions between cultured specimens. Hair *et al*. (2004) also tested the suitability of growing collected shrimps individually in 200 ml clear plastic jars *in situ*, which had previously been drilled to facilitate water flow but minimize food loss. Shrimps being cultured in individual plastic jars were provided with the same diet used for communal rearing, although they were only fed once a day since individual feeding of cultured shrimps was found to be very labour intensive. The use of transparent jars promoted algal growth on the shrimp's exoskeleton, with specimens fouled with algae commonly losing their appendages or dying during ecdysis. However, by simply painting the jars black in order to reduce light intensity, survival of cultured shrimps was significantly improved (Hair *et al*., 2004). Whereas cultured *S. hispidus* were ready for export only after 4 weeks of grow-out, with an average body size of 25 mm, *S. zanzibaricus* and *S. tenuirostris* did not reach export size, even after 2 months of grow-out. These experimental trials reinforce the importance of understanding species-specific nutritional requirements, since even closely related species may display significantly different dietary needs.

Apart from crest nets, the collection device CARE has already been shown to be a suitable way to collect several species of post-larval marine ornamental decapods, as well as spiny lobsters (Lecaillon, 2004). Several different types

of larval decapods are also known to settle on spiny lobster puerulus collectors (Mills & Crear, 2001). The idea of harvesting spiny lobster pueruli from the wild to be grown in captivity to a marketable size has gained considerable impetus in recent years (Phillips & Evans, 1997; McVeigh, 2002). Curiously, this culture approach for spiny lobsters has started to be addressed in relation to larval culture bottlenecks similar to those affecting marine ornamental shrimp culture, namely: unsuitability of 'typical' larval diets; long larval development periods; and poor survival to metamorphosis of cultured larvae. It will not therefore be surprizing if the commercial harvest of newly settled marine ornamental shrimps, using puerulus collectors, also starts to be considered. In certain locations, the capture of newly settled marine ornamental shrimps may be a valuable 'by-catch' of spiny lobster puerulus collection, significantly increasing the profitability of this activity. Marine ornamental shrimp species sharing the same habitats as spiny lobsters (e.g. *Stenopus* and *Lysmata* species also occupy rock crevices or reef ledges) may eventually be more readily collected. It is obvious that not all species will display the same potential to settle in these structures. As an example, alpheid shrimps, which show certain affinities towards specific types of substrate, as well as shrimp species living in association with other invertebrates, may not settle in these structures. It may also be expected that because of the highly territorial nature of some species (e.g. *Stenopus*) only a limited number of specimens will settle per collecting device. Given the large number of sampling devices already developed for puerulus collection, for either scientific or commercial purposes (Jernakoff, 1990; Booth *et al.*, 2001; Mills & Crear, 2004), it is possible that a particular design allows the settlement of a larger number of marine ornamental shrimps. For preliminary trials, the bottlebrush collector may be the ideal choice. These types of collector are built from cheap and lightweight materials and have proven to be cost-effective, robust and easy to handle for the collection of the puerulus stage of the spiny lobster *Jasus edwardsii* (Hutton, 1875) (Mills & Crear, 2004). According to these authors, each collector comprised 10 'rosettes'. These were made from a 1.8 m $\times$ 0.4 m strip of black windbreak mesh, folded concertina-style and pinched at its centre to form a rosette approximately 0.4 m diameter. The rosettes were fastened to a 0.5 m length of 15 mm diameter PVC pipe using cable-ties at 50 mm intervals. The areas within rosettes as well as among them formed appropriate shelters for pueruli. A 200 mm diameter Styrofoam float is tied to the top of the PVC pipe and a mooring rope with swivel to the bottom. After hauling, settled pueruli are easily recovered by vigorously shaking bottlebrush collectors in a 250 liter plastic bin. By being lightweight, these collectors can be easily adapted to long-line deployment and a small vessel may be employed during regular monitoring and hauling.

If a specific collector design must be perfected, or even fully redesigned, to allow commercial-scale collection of newly settled marine ornamental shrimps, the highest priority during this process must be its cost effectiveness.

A collector's effectiveness must invariably address its construction, deployment and servicing costs. The three steps approach described by Mills & Crear (2004) for the development of puerulus collectors may also be used to address the suitability of devices to be employed in the collection of newly settled marine ornamental shrimps:

(1) evaluate the use of inexpensive and readily available materials, which may provide a suitable settlement substratum (e.g. empty oyster shells, discarded trawl meshes, wind-break meshes and fruit sack meshes);
(2) test the performance of candidate materials in field trials using comparable deployment methods; and
(3) build collectors using the best performing materials and compare their catch rates in field trials.

Minimizing the loss rate of newly settled specimens from collectors during hauling is also of vital importance to maximize the profitability of these devices. If newly settled marine ornamental shrimps cling strongly to the substrate when the collectors are hauled, as has been recorded for the spiny lobster pueruli (Booth *et al*., 1991), escape of settled specimens during hauling will not be an issue.

Although the collection of spiny lobster pueruli for subsequent grow-out already supports commercial-scale production (Tuan *et al*., 2000; Thuy & Ngoc, 2004), it has been widely opposed by those engaged in spiny lobster fisheries since there is a generalized belief that this practice will negatively affect wild stocks. A study estimating the survival of juvenile *Panulirus cygnus* in Western Australia indicated that natural mortality occurring in the first year is density dependent (Phillips *et al*., 2003). According to the authors, under this scenario, puerulus collection will probably have a negligible impact on wild fisheries. However, since this study only addressed a single species and species-specific variations in juvenile survival may occur, lobster fishery managers have adopted measures to reach 'biological neutrality' (Gardner *et al*., 2006). This approach requires a compensatory process to balance increased 'fishing mortality' induced by the collection of pueruli. Two different approaches were considered for achieving biological neutrality: operating through a quota lease system; and a 'reseeding' system, returning a number of juveniles to coastal reef areas after 1 year of grow-out, in order to compensate for potential negative effects that puerulus collection may have had 1 year earlier in wild populations.

The rationale used to support the concerns expressed towards the sustainability of spiny lobster puerulus harvest may also be valid for the collection of newly settled marine ornamental shrimps. If the harvest and grow-out of newly settled marine ornamental shrimps starts to be addressed on a commercial scale, the management approaches proposed for spiny lobster puerulus collection may be a good starting point to develop sustainable practices for

this new activity. Understanding juvenile mortality in wild populations during the first year for the most heavily collected species will be crucial to develop reliable management models. According to Gardner *et al.* (2006), assuming a conservative value of first-year survival for wild populations may have the effect of increasing the operational costs to aquaculture enterprises. None the less, it will also increase wild stocks under the management practice of 'biological neutrality'. If using a quota lease approach, the use of a conservative survival estimate will remove a higher quota from the fishery than that actually required to compensate for the harvest of newly settled specimens. If the reseeding policy is employed also using a conservative survival estimate, it may lead to the release of more juveniles after 12 months than those required to compensate for the previous harvest of newly settled specimens. As pointed out by Gardner *et al.* (2006), if a conservative survival estimate is used, both management systems have the potential to act as reliable tools to increase the stocks of wild populations while simultaneously allowing the development of an alternative aquaculture industry. The authors also stress that a reseeding approach will be favoured over the quota lease when:

(1) wild survival is low and culture survival is high;
(2) wild survival is high and cost to lease quota is high; and
(3) wild survival is high and cost for grow-out feeds is low.

8.4 Packing, shipping and acclimatizing ornamental shrimps

The trade in marine ornamental species relies heavily on the transport of live specimens for more or less prolonged periods of time. Although traded specimens may sometimes be transported by land, namely during retail sales to hobbyists or wholesale activities in relatively small geographical areas, most commercial marine ornamental species transactions depend on aerial transportation. The International Air Transport Association (IATA) provides the Live Animal Regulations, which are enforced by the European Union and the US Fish and Wildlife Service and generally meet or exceed the intent of the US Animal Welfare Act (AWA). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the World Organization for Animal Health (OIE) also officially recognize these regulations for the transport of animals by air (IATA, 2007). Specimens being traded must be packed according to these regulations and fully respect international safety measures and animal welfare.

Marine ornamental shrimps about to be shipped are commonly placed under starvation 24 hours before being packed. If shrimps are being stocked together, as when performing communal rearing, it is advisable that during this period the number of shelters available per tank is increased or stocking density decreased. The main goal of this procedure is to decrease the chances

of starved shrimps cannibalizing conspecifics. Shrimps should be collected with the help of hand nets with a small mesh size (cloth-like), in order to prevent body appendages or spines from being tangled and eventually damaged. Prior to packing, shrimps are generally placed in a container for about 10 minutes where they are allowed to excrete metabolic compounds resulting from handling stress (Hunter & Uglow, 1993; Paterson *et al.*, 2005). Only after this period should shrimps be gently placed in the shipping container, commonly a plastic bag. This simple procedure delays the deterioration of shipping water quality before specimens are actually being shipped. Water quality issues are more likely to occur in a densely packed shipping container, although higher packing densities significantly reduce transportation costs. In this way, an ideal balance of conditions for cost-effective shrimp shipping must be achieved without increasing the risks of mortality. Despite their relatively small size, marine ornamental shrimps are commonly packed individually. Although this approach is known to be more time consuming, as well as more expensive, packing several shrimps in the same container significantly increases the risks of mortality. For some species, such as *Stenopus* spp. and pair-living *Lysmata* spp., multi-packing is not even an option due to the high levels of intraspecific aggression. Even when packing breeding pairs, which have already been together for a long period of time, packing each shrimp in an individual container is always preferred over packing them together. The stress induced during shipping is commonly enough to disrupt the pair bond and trigger agonistic behaviors, which invariably leads to the death of one of the specimens. In order to allow shipped breeding pairs to be re-established, the containers holding each member of the pair are usually connected and shipped together. It is not unusual to record shrimps moulting during the shipping period. The occurrence of this event is not important for mono-packed shrimps. However, if multi-packing is employed, even with a reduced number of specimens, it is likely that newly moulted shrimps will rapidly be cannibalized by previously starved conspecifics. The decaying remains of dead shrimps, as well as the excretion products of conspecifics feeding on them, will significantly decrease water quality and in extreme situations cause the death of all specimens packed in the same container.

When shipping marine ornamental species, it is very important to know in advance the duration of the transportation period. This period will define for how long shipped shrimps will remain inside their containers before any action can be taken to re-establish water quality. Average transportation periods will range between 24 and 48 hours. However, in extreme situations, mainly due to flight delays or customs clearance bureaucracy, 72 or more hours may elapse between packing and reception in the customer's facilities. After 48 hours of shipping the risks of mortality significantly increase, although cultured marine ornamental shrimps may be more tolerant of these extreme situations than wild specimens. A remarkable example of such resilience was recorded with captive-cultured juvenile Monaco shrimps *L. seticaudata* (approximately

30 mm of total body size). These specimens were individually stocked in plastic bags in 50 ml of synthetic sea water with an oxygen saturated atmosphere and packed in a 15 mm thick Styrofoam box. A delay in a flight connection meant these specimens underwent an 84-hour shipping period. Despite the extended duration of transportation and the sharp decrease in water quality and temperature, all shrimps survived (Calado, unpubl. obs.).

Live shrimps, as well as other marine ornamental species (Cole *et al*., 1999) traded in the marine aquarium industry are commonly packed in plastic bags provided with one-third of marine water to two-thirds pure oxygen. Sometimes, double bags are used to minimize the risks of water leaks during shipment, which may not be uncommon in bags holding species with strong chelipeds or exhibiting protruding body spines. It is also a common practice to introduce a small piece of mesh in the bag, allowing packed shrimps to cling to it during the shipping period. Apparently, clinging shrimps are less susceptible to the stress induced by abrupt movements of the packing container during transportation. Plastic bags with rounded bottoms are usually preferred over traditional plastic bags, since they decrease the risks of shrimps being trapped in the folds located in the bottom of traditional plastic bags once these are stocked in boxes. Square-bottomed bags are also a good alternative for live shrimp transportation since, after being placed in the bottom of a shipping box, they utilize its entire area to maximize air/water contact and ensure maximum oxygen transfer during the entire travelling period. The addition of pH buffers or ammonia removers to shipping water, a fairly common practice when shipping marine ornamental fishes (Cole *et al*., 1999), is not usually employed when shipping ornamental shrimps. Plastic bags are usually sealed with rubber bands or metal clips. The first option is usually more reliable to prevent leaks, although it is also more time consuming. After being properly sealed, the bags are placed inside Styrofoam boxes, which provide temperature insulation and protection from physical damage during shipping. During winter (or summer) shipment, the use of heat (or cold) packs may be necessary to prevent water temperature from dropping (or increasing) to dangerous values. In both scenarios these packs are commonly attached to the inner surface of the cover of the Styrofoam box and should never be in direct contact with the shipping bags. The Styrofoam box is then placed inside a cardboard box that must display the international symbols required by IATA (e.g. 'This side up' and 'Live specimens' symbols).

It is known that different decapod crustaceans exhibit variable spectral sensitivities (Cronin, 1986; Johnson *et al*., 2002).The use of dim red lights during the packing process for marine ornamental shrimps decreases their escape behavior (e.g. tail flips), which is commonly observed under white fluorescent lights. The minimization of handling stress prior to shipping significantly increases the chances of packed shrimps surviving.

During the shipping period, the water quality in closed plastic bags may dangerously decrease. Water may become oxygen-depleted and accumulate

excessive carbon dioxide, leading to a reduction in pH. The metabolic activity of packed specimens can also increase ammonia levels in the water, which may be lethal if shipping lasts for a longer period than initially planned.

Ammonia (NH_3) and ammonium ion (NH_4^+) are two chemical species which exist in a dynamic balance in seawater ($NH_3 + H^+ \leftrightarrow NH_4^+$). High pH values promote the existence of the un-ionized form (NH_3), which is also the most toxic (Mead, 1985). The decrease in water pH during shipping, induced by the increment in carbon dioxide levels, allows accumulated ammonia to occur in its less toxic form, the ammonium ion (NH_4^+). When unpacking marine ornamental shrimps it is important to ensure that during acclimatization neither pH nor temperature will be rapidly increased. Apart from inducing osmotic and thermal stress, a rapid increase in pH and/or temperature promotes the transformation of the ammonium ion (NH_4^+) to the most toxic un-ionized form of ammonia accumulated in the shipping water (NH_3) (Cole *et al*., 1999). This fatal mistake usually takes place when air stones are employed to increase the levels of dissolved oxygen in shipping water, since this procedure also promotes a sharp decrease in carbon dioxide content, indirectly promoting an increase in pH. For this reason one possible solution to acclimatize newly arrived shrimps correctly should include the following steps:

(1) Unpack newly arrived shrimps under dim red light in order to decrease stress (e.g. abrupt escaping behavior through tail-flip movements).
(2) Use 1 m long × 1 m wide × 0.1 m high reception tanks equipped with heaters. The tanks must also be equipped with an inflow connected to the system that will hold the shrimps after acclimatization and an outflow connected to the drain system of the facility, which collects the water that will no longer be recirculated.
(3) Place all received shrimps, including their shipping water, inside the reception tanks.
(4) Be sure to have individual containers inside reception tanks when unpacking aggressive species (e.g. *Stenopus* spp.).
(5) Open the reception tank inflow to enable a continuous fast drip flow and allow the water from the holding system to slowly replace the shipping water (excessive water will exit through the reception tank outflow).
(6) Use the reception tank heaters to adjust water temperature (although inflowing water will also help to slowly adjust water temperature to the value in the holding system).
(7) Allow a total volume of inflowing water to enter the reception tank that is equal to at least twice its volume (two full water renovations).
(8) If a large number of specimens are present in the reception tanks, air stones may be used to increase oxygen levels, but only after one full renovation by inflowing water from the holding system.
(9) Use a hand net with a small-sized mesh (cloth-like) to gently remove acclimatized shrimps to the holding tanks.

As stressed by Cole *et al.* (1999), even the most effectively run marine ornamentals production enterprise will never succeed if packing and shipping procedures are neglected. It is vital to identify and minimize any potential risks recorded during these processes, as well as when receiving new shrimp broodstock or juveniles. In order to ensure profitability, cost-effective solutions must be designed to fulfil species-specific requirements as well as longer shipping times. The use of an effectively designed packing room must allow shrimps to be properly harvested and prepared well in advance of shipping deadlines. If possible, the same room must also be prepared to receive newly imported shrimps and ensure that acclimatization protocols can be successfully put into practice. Minimizing the risks of mortality before, during and after shipping is essential for long-term success of any commercial operation dealing with marine ornamentals.

Chapter 9

Other Marine Ornamental Decapod Species with Culture Potential

Although caridean and stenopodidean shrimps are the most popular ornamental decapods in the marine aquarium trade (Calado *et al.*, 2003c), several other species belonging to different decapod groups are also widely traded. In fact, the growing demand for these 'non-shrimp' species in the hobby sector has led traders and researchers to widen their scope when selecting ornamental decapods with culture potential.

9.1 Marine ornamental porcelain crabs

According to Debelius (2001), porcelain crabs received this unusual popular name because of their porcelain-white body and their tendency to break easily (e.g. they autotomize their chelipeds when stressed). Although at first glance porcelain crabs may look similar to true crabs, these organisms belong to the infraorder Anomura MacLeay, 1838, being systematically closer to hermit crabs. They are members of the family Porcellanidae Haworth, 1825, and can be easily distinguished from true crabs by the presence of a reduced fifth pair of pereiopods. These appendages act as grooming limbs and are equipped with different types of grooming setae and smooth sensillae to locate and identify fouling objects. They are extremely flexible and reach most parts of the crab's body, including the gill chamber (Fleischer *et al.*, 1992). Porcelain crabs also display considerably longer antennae than true crabs and their third pair of maxillipeds is largely modified for filter feeding. These organisms have the remarkable ability to change their feeding mechanism according to water flow conditions (Trager & Genin, 1993). When in still water and slow flow, they actively sweep the water with their maxillipeds. However, when flow speed increases, they switch from active suspension feeding to passive feeding. When facing a low frequency oscillating flow, the crabs rhythmically reorient their suspension feeding fans, placing the maxillipeds upstream. If porcelain crabs are exposed to a high-frequency oscillating flow, they perform

alternate movements of their third maxillipeds so that one fan is extended and the concave face is directed upstream while the other is withdrawn (Achituv & Pedrotti, 1999).

Although several species of porcelain crab in the genus *Petrolisthes* Haig, 1962, and *Porcellana* Lamarck, 1801, display dazzling colorations, their cryptic habits make them less appealing to the marine aquarium hobby. The most popular porcelain crab species are *Neopetrolisthes ohshimai* Miyake, 1937, and *Neopetrolisthes maculatus* (H. Milne Edwards, 1837). Both species occur in association with large sea anemones (e.g. *Entacmaea* Ehrenberg, 1834 and *Stichodactyla* Brandt, 1835), commonly as mated pairs and with females usually reaching larger sizes than males. Both species can be easily distinguished by their body coloration, with *N. ohshimai* exhibiting large red dots scattered through the carapace and chelipeds whereas *N. maculatus* shows numerous small pink or violet dots.

So far, porcelain crabs have not been produced in captivity. These organisms display a reduced number of larval stages (two zoeae and one megalopa) (Table 9.1) (Fujita & Osawa, 2003), which means that under optimal conditions their larval development will probably take less than 10 days. Among other features (Figure 9.1), the two zoeal stages can be distinguished by the number of setae in the exopods of the first and second maxillipeds, with the first zoeal stage only displaying four setae (Lebour, 1943). Larvae can be fed newly hatched *Artemia* nauplii, although the inclusion of rotifers until all specimens have moulted to the second zoeal stage may significantly improve larval survival. Providing the megalopa with enriched *Artemia* metanauplii and/or minced pieces of squid or mussel gonad will certainly increase the rate of specimens successfully moulting to the first crab instar.

The short larval development of porcelain crabs makes them good aquaculture candidates. However, because of their associative behavior with sea anemones, it is possible that settlement cues released by the host species may promote the settlement of *Neopetrolisthes* megalopa. From a technical perspective, the solutions described earlier to promote the settlement of partner shrimps *Periclimenes* larvae, which also associate with sea anemones, may also be used during the larval culture of *Neopetrolisthes*. The zoeal stages of porcelain crabs exhibit an impressive rostrum, which sometimes may be up to four times longer than the carapace, as well as two long spines in the posterior region of the carapace (e.g. Lebour, 1943; Fujita & Osawa, 2003). As for larval marine ornamental shrimps in the genera *Lysmata* and *Stenopus*, the larval features of zoeal porcelain crabs require specially designed larval culture systems to avoid damaging these structures. The culture system described by Calado *et al.* (2003a) may be used to raise these frail larval stages, although it will probably be necessary to add extra surfaces to the culture tank (e.g. by adding extra artificial grass strips) to allow cultured megalopa to cling to them.

Despite being filter feeders, the husbandry of *Neopetrolisthes* broodstock is not particularly difficult since, at least in captivity, they readily capture

Table 9.1 Families, scientific and common names, world distribution and larval development of ornamental decapods (excluding caridean and stenopodidean shrimps) with culture potential

Family	Species	Common name	Distribution	Larval development
Porcellanidae	*Neopetrolisthes maculatus*	Porcelain crab	Indo-Pacific	2 zoeal stages + 1 megalopa[1]
	Neopetrolisthes ohshimai	Porcelain crab	Indo-Pacific	2 zoeal stages + 1 megalopa [2]
Diogenidae	*Calcinus elegans*	Electric-blue and pumpkin hermit crab	Indo-Pacific	5 zoeal stages + 1 megalopa[3]
	Calcinus laevimanus	Blue-eyed hermit crab	Indo-Pacific	5 zoeal stages + 1 megalopa [2]
	Calcinus tibicen	Red legs hermit crab	Western Atlantic	5 zoeal stages + 1 megalopa [3]
	Clibanarius erythropus	Green legs hermit crab	Eastern Atlantic	4 zoeal stages + 1 megalopa [4]
	Clibanarius tricolor	Blue legs hermit crab	Western Atlantic	4 zoeal stages + 1 megalopa [2]
Inachidae	*Stenorhynchus seticornis*	Arrow crab	Western Atlantic	2 zoeal stages + 1 megalopa [5]
Mithracidae	*Mithraculus forceps*	Ruby crab	Western Atlantic	2 zoeal stages + 1 megalopa [6]
	Mithraculus sculptus	Emerald crab	Western Atlantic	2 zoeal stages + 1 megalopa [7]
Plagusiidae	*Percnon gibbesi*	Sally Lightfoot	Atlantic and Eastern Pacific	6 zoeal stages + 1 megalopa [8]
Xanthidae	*Lybia tessalata*	Pom-pom crab	Indo-Pacific	4 zoeal stages + 1 megalopa [9]
Trapeziidae	*Trapezia* spp.	Coral crab	Indo-Pacific	4 zoeal stages + 1 megalopa [9]
Tetraliidae	*Tetralia* spp.	Coral crab	Indo-Pacific	4 zoeal stages + 1 megalopa [10]
Palinuridae	*Panulirus versicolor*	Blue spiny lobster	Indo-West Pacific	Unknown [10]
	Justitia longimanus	Long handed spiny lobster	Indo-Pacific and Western Atlantic	Unknown [11]
Enoplometopidae	*Enoplometopus daumi*	Daum's reef lobster	Indo-West Pacific	7 zoeal stages[12]
	Enoplometopus debelius	Debelius's reef lobster	West and Central Pacific	7 zoeal stages[12]
	Enoplometopus occidentalis	Red reef lobster	Indo-Pacific	7 zoeal stages[12]

[1] Fujita & Osawa, 2003.
[2] Assumed according to the stages displayed by other species in the genus.
[3] Provenzano, 1962.
[4] Bartilotti *et al.*, in press.
[5] Yang, 1976.
[6] Wilson *et al.*, 1979.
[7] Rhyne *et al.*, 2006.
[8] Paula & Hartnoll, 1989.
[9] Assumed according to the stages displayed by other xanthoid genus.
[10] Johnson (1971) and Michel (1971) provide some information on the phyllosoma of this species.
[11] Robertson (1969) and Johnson & Robertson (1970) provide some information on the phyllosoma of this species.
[12] G. Penha-Lopes, pers. comm.

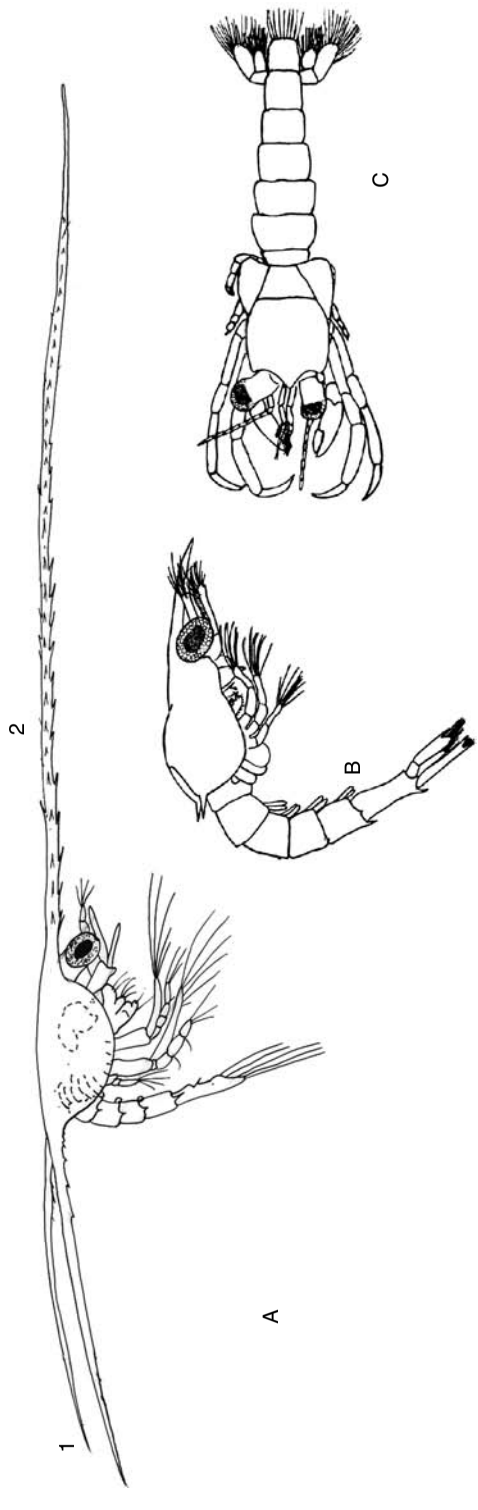


Figure 9.1 'Typical' porcellanid zoea (A), diogenid zoea (B), and glaucothoe [a common designation for the megalopa of hermit crabs (C)]; commonly damaged larval appendages during culture: posterior carapace projections (1) and the extremely elongated rostrum (2) (larvae not to scale) [adapted from Lebour (1943) and Provenzano (1962)].

suspended food particles and also consume anemone mucus and scraps of food (Sprung, 2001). When stocking reproductive pairs it may also be necessary to place their symbiotic sea anemones inside the maturation tank since *Neopetrolisthes* tends to display poor survival rates in the absence of the host (Calado, unpubl. obs.). This procedure requires the use of highly efficient larval collectors in order to decrease the risks of newly hatched larvae being captured by the host sea anemone. The grow-out period of juvenile *Neopetrolisthes* may also be marked by intraspecific aggression and territoriality, as recorded for other anemone-dwelling porcelain crabs (e.g. Baeza *et al.*, 2002). This assumption is based on the fact that, like other symbiotic decapod crustaceans, these organisms manipulate the use of their host (Thiel & Baeza, 2001). The presence of host anemones in grow-out tanks may also play a crucial role for the culture of juvenile porcelain crabs. However, Baeza & Stotz (2001) have recorded that host selection may change during the ontogeny of the porcelain crab *Allopetrolisthes spinifrons* (H. Milne Edwards, 1837), with newly settled organisms using non-anthozoans as alternative hosts as a result of the limited availability of sea anemones.

9.2 Marine ornamental hermit crabs

Hermit crabs belong to the infraorder Anomura and some species have become highly popular in the marine aquarium trade. Several hermit crabs, as well as snails and brittle star species, are available in the trade under the popular name of 'cleaning crews' (Figure 9.2). Most hobbyists use these organisms as reef aquarium janitors, which are commonly employed to control the growth of nuisance algae, to stir the superficial layers of sandbeds in reef displays and to eat food leftovers (Sprung, 2002). Although a wide variety of hermit crab species are commonly available in the aquarium trade, the members of the family Diogenidae Ortmann, 1892 are certainly the most popular ones. Species from genus *Clibanarius* Dana, 1852 and *Calcinus* Dana, 1851 have become top choices among hobbyists, mainly because they are reef-safe and highly efficient in algae control. Hermit crabs commonly occur in large aggregations over reef flats, lagunar areas and rocky intertidal zones (Gherardi & Benvenuto, 2001), which facilitates the capture of hundreds of organisms in a reduced amount of time (e.g. Calado & Dinis, in press). The considerable increase in the demand for these organisms may be pushing wild populations to the limits. As an example, the small size displayed by hermit crabs harvested in Florida and recently imported to Europe indicates that a significant fishing effort is affecting organisms only a few weeks after settlement, most probably before reaching sexual maturity (Calado, unpubl. obs.). According to their availability in the marine aquarium trade, the most heavily collected hermit crab species appear to be the electric blue and pumpkin hermit *Calcinus elegans* (H. Milne Edwards, 1836), blue-eyed hermit *C. laevimanus* (J. W. Randall,

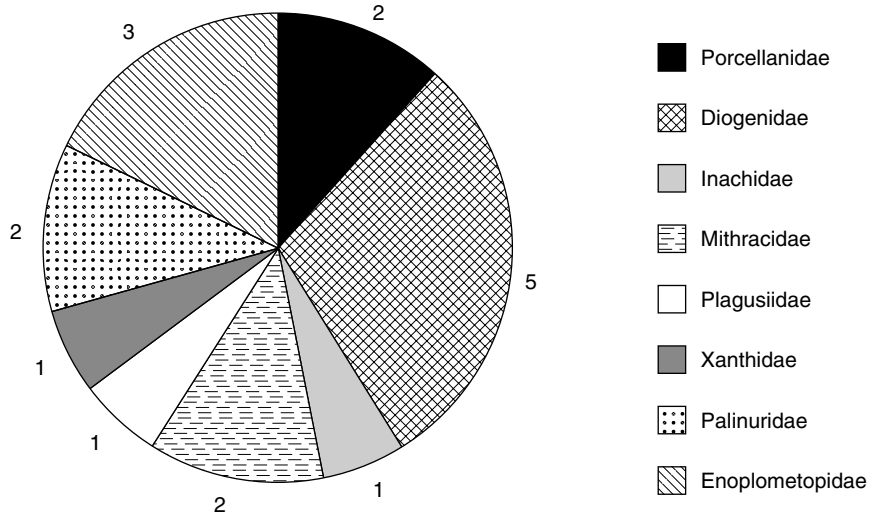


Figure 9.2 Decapod marine ornamental species (excluding shrimps and coral crab families Trapeziidae and Tetraliidae) displaying good culture potential.

1840), the red-legs hermit *C. tibicen* (J. F. W. Herbst, 1791), the green-legs hermit *Clibanarius erythropus* (Latreille, 1818) and the blue-legs hermit *C. tricolor* (Gibbes, 1850) (Table 9.1).

Although it is possible to mature hermit crabs in captivity, the most common approach is to stock wild ovigerous females until larval hatching in maturation tanks similar to the ones used for marine ornamental shrimps (Calado *et al.*, 2007c). Body size is known to influence embryo production in hermit crabs (Mantelatto & Garcia, 2000; Contreras-Garduño & Córdoba-Aguilar, 2006). However, shell architecture can also significantly affect that reproductive trait (Fotheringham, 1980; Bertness, 1981), as well as the mating behavior of hermit crabs (Hazlett & Baron, 1989). Ziegler & Forward (2006) have also verified that *Clibanarius vittatus* (Bosc, 1802) ovigerous females displayed stereotypical hatching behaviors but only when occupying intact shells. The female stands on the walking legs and thrusts the abdomen, moving the shell in an oscillating motion. The authors suggest that this movement may assist in breaking the embryos' outer membrane. These females generate a water current inside the shell with the scaphognathite, as well as the mouthparts, forcing newly hatched larvae out of the female's shell. In contrast, females in damaged shells do not display such behaviors, larval release becoming a prolonged event with little movement of the female and negatively affecting larval viability. Therefore, large ovigerous hermit crab females occupying intact shells, whose architecture maximizes egg production, should be selected as broodstock when performing captive culture trials. The opacity of the shells occupied by hermit crabs makes difficult the determination of sex (Figure 9.3) and the detection of ovigerous females. However, a simple way to detect the presence of specimens

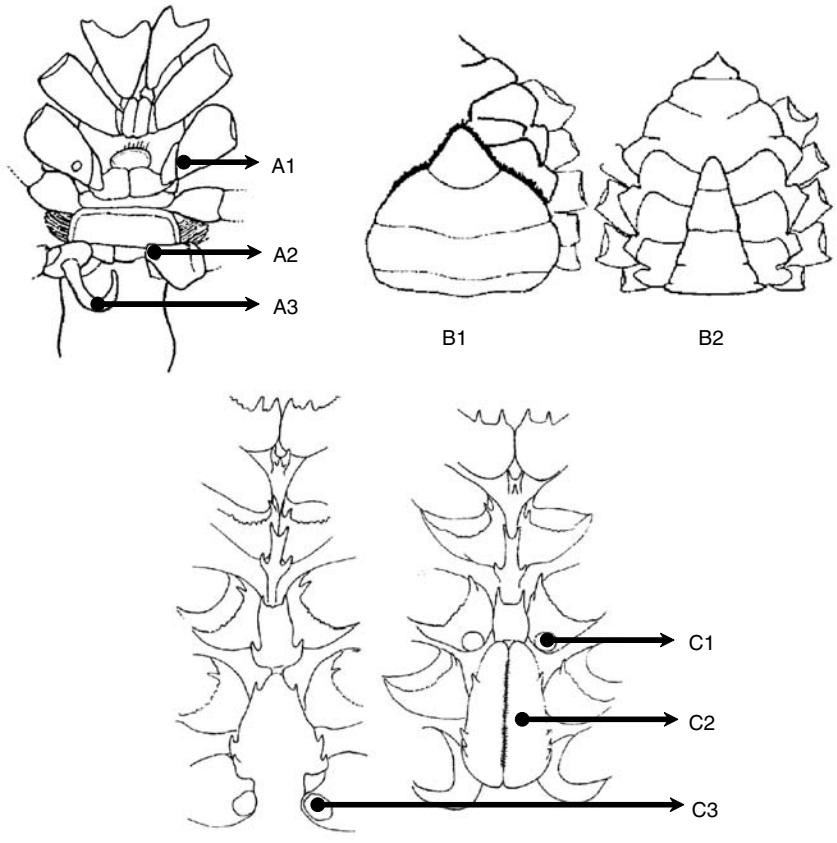


Figure 9.3 Diagnosing sexual features of hermit crabs: A1, female gonopore (or genital opening) located in the coxa of the third pair of pereiopods; A2, male gonopore (or genital opening) located in the coxa of the fifth pair of pereiopods; A3, male coxal tube. Diagnosing sexual features of true crabs: B1, female's abdomen; B2, male's abdomen. Diagnosing sexual features of true lobsters: C1, female gonopore (or genital opening) located in the coxa of the third pair of pereiopods; C2, female's receptaculum seminis (where the male's spermatophore is deposited during copulation); C3, male gonopore (or genital opening) located in the coxa of the fifth pair of pereiopods. Drawings are not to scale.

incubating embryos is to place them on a horizontal surface and hold the shell with its aperture facing up. After a few seconds, in an effort to regain their normal position, the hermit crab will partially crawl out of its shell and expose its anterior abdominal region. At this moment developing embryos, if present, will be perfectly visible.

The larval development of *Clibanarius* species is characterized by four (sometimes five) zoeal stages plus one megalopa (Pike & Williamson, 1960; Siddiqui *et al*., 1993; Bartilotti *et al*., in press), whereas *Calcinus* species display five (sometimes six) zoeal stages plus one megalopa (Pike & Williamson, 1960; Provenzano, 1962). Their zoeal stages can be differentiated by the

analysis of morphological features similar to those used to stage caridean shrimp larvae (Figure 9.1). The megalopa of hermit crabs is sometimes termed the glaucothoe. The zoeal stages of both genera can be easily cultured in larval rearing tanks for marine ornamental shrimps and by using newly hatched and/or enriched *Artemia* metanauplii as larval prey. Hermit crabs cultured in captivity, under suboptimal conditions, are known to display extra zoeal stages, leading to an extended larval development (Provenzano, 1962). After a few days, depending on hermit crab species, cultured megalopa change their swimming behavior, becoming less active swimmers, and start seeking gastropod shells near the bottom of the culture tank (Reese, 1968; Harms, 1992). The main bottleneck for the larval culture of marine ornamental hermit crabs is related to the moult from megalopa to juvenile. The megalopa 'shell entering' behavior is elicited through tactile stimuli from mechanoreceptors on chelipeds and pereiopods, which trigger a sequential series of fixed motor patterns (Reese, 1963; Hazlett, 1971). In this way, in the absence of suitable gastropod shells cultured megalopa may significantly delay metamorphosis (Harms, 1992). Curiously, Blackstone & Joslyn (1984) suggest that hermit crabs probably display a flexible preference concerning gastropod shells at metamorphosis, since this behavioral plasticity would give them an advantage over specimens programmed to search for a specific shell type.

Cylindrico-conical culture tanks cannot be used during this culture period unless small horizontal surfaces (e.g. Petri dishes) are suspended inside them in order to allow gastropod shells to be available for developing megalopa. Cylindrico-spherical tanks seem to be a better option, since gastropod shells can be readily offered to developing megalopa by placing them on the less sloping tank bottom areas (Calado, unpubl. obs.). Ensuring a regular supply of shells that display appropriate architecture and size for hermit crab megalopa in order to prevent the delay of metamorphosis, is a difficult task. Apart from being a time-consuming operation, the regular supply of suitable gastropod shells is unlikely to be compatible with commercial-scale production of these organisms. Even if an enterprise manages to overcome the settlement bottleneck described, it will face another challenge during grow-out since optimal juvenile growth will only be achieved if increasingly larger gastropod shells are periodically offered. Gilchrist (1985) demonstrated that the type of shell occupied by juvenile hermit crabs influences their future shell preferences. Providing shells with inadequate architectural features (e.g. length/width ratio, shell aperture shape, damage and encrustation levels) and/or in insufficient numbers will trigger agonistic behaviors related to shell dislodging between cultured organisms (Hazlett, 1968, 1972, 1987).Unsuitable shell size also affects body growth since a hermit crab may only grow if it moves to a larger shell. If larger shells are not available, the individual has to suppress growth to the maximum value allowed by the shell it occupies (Sato & Seno, 2006). Nevertheless, it is important to point out that in natural

populations most hermit crabs are commonly recorded occupying suboptimal shells (Vance, 1972).

Male and female hermit crabs from the same species may exhibit different shell preferences (Benvenuto & Gherardi, 2001; Yoshino & Goshima, 2002), requiring an even greater supply of gastropod shells during grow-out. Additionally, the optimal shell type may not be that of the most abundant gastropod in the area inhabited by the cultured hermit crab species (Orians & King, 1964). A potential solution to facilitate the tedious task of sorting gastropod shells with distinct architectural features is to culture in the same grow-out tank different species of hermit crabs, which are known to exhibit distinct shell preferences. Sympatric hermit crab species may be suitable candidates for such polyculture trials, since they share a common resource (available shells) in their natural habitat (Turra, 2003).

Immediately before reaching a commercial size, cultured specimens can be supplied with shells covered by coralline algae and/or small pieces of soft corals, namely pulsing *Xenia*. Hermit crabs occupying such shells, commonly designated in the hobby as 'fancy shells', always reach higher market values than specimens occupying regular shells.

Unless creative solutions are employed to eliminate, or at least decrease, the labour-intensive activity associated with periodic shell supply during hermit crab culture, the final production cost per specimen may be too high to compete with the low unitary value of wild ones. Hermit crabs are the perfect example of how profitability can negatively affect conservation efforts concerning the captive culture of marine ornamental decapods that are heavily collected in the wild (Calado & Narciso, 2003b).

9.3 Marine ornamental crabs

True crabs belong to the infraorder Brachyura Latreille, 1802. The popularity of a small number of species has significantly increased among marine aquarium hobbyists in recent years (Table 9.1). True crabs, namely those in the genera *Mithraculus* White, 1847, and *Percnon* Gistel, 1848, are common members of reef aquarium 'cleaning crews', being commonly employed to control the growth of nuisance algae (Figure 9.2) (Sprung, 2002). Brachyuran crabs can be easily sexed by the shape of their abdomen (see Figure 9.3 for diagnosing sexual features).

Despite their popularity, some hobbyists do not consider true crabs as reef safe and do not advocate their use in reef displays. The arrow crab *Stenorhynchus seticornis* (J.F.W. Herbst, 1788), belongs to the family Inachidae MacLeay, 1838, and is a well-known example of a popular crab species labelled as 'reef safe' that tends to prey upon other invertebrates, and even small fishes, in reef aquariums (Fosså & Nielsen, 2000; Sprung, 2001). Like all members of the superfamily Majoidea Samouelle, 1819, widely known as spider crabs,

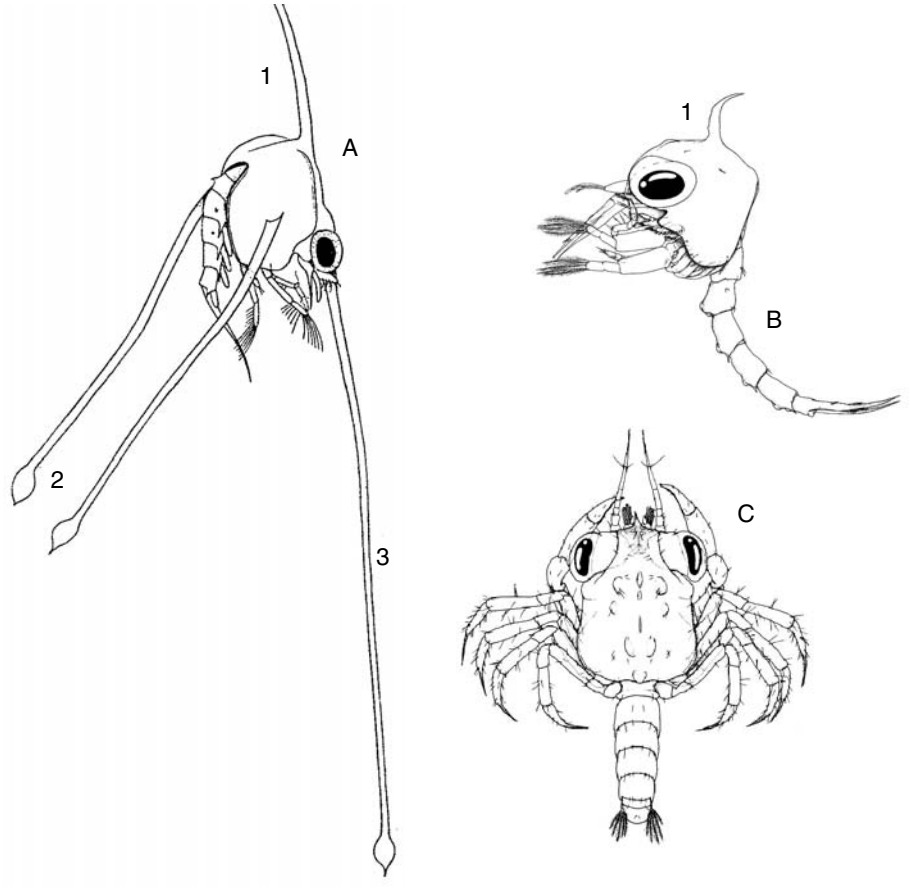


Figure 9.4 'Typical' *Percnon* zoea (A), majid zoea (B) and megalopa (C); commonly damaged larval appendages during culture: dorsal carapace spine (1), carapace projections (2) and the extremely elongated rostrum (3) (larvae not to scale) (adapted from Gurney, 1942).

it displays a relatively short larval development: two zoeal stages plus one megalopa (Figure 9.4) (Pohle & Marques, 2000). For this reason the larval culture of *Stenorhynchus* Lamarck, 1818 species in captivity may not be a bottleneck, since newly hatched larvae readily accept *Artemia* nauplii (Yang, 1976; Paula & Cartaxana, 1991). Additionally, females of *S. seticornis* are ovigerous year round (Cobo, 2002), so it is possible to get access to high quality 'wild larvae' during the entire year through the collection of wild ovigerous females. Inducing maturation in captivity is also possible by mimicking abiotic conditions in the wild and by providing a varied diet (e.g. commercial frozen mixes for reef aquariums appear to be cost-effective solutions). None the less, the quality of larvae produced in captivity is always inferior to that of wild larvae (Calado, unpubl. obs.). Communal rearing of *Stenorhynchus* juveniles will certainly represent a greater challenge than its larval culture, since male

specimens tend to display strong agonistic behaviors towards conspecifics of the same sex as a result of mating competition (Debelius, 2001).

Considering popularity and commercial scale culture viability, majoid crabs of the genus *Mithraculus* are certainly a better choice than *Stenorhynchus*. This crab genus belongs to the family Mithracidae Balss, 1929. It is closely related to the genus *Mithrax* Desmarest, 1823, and older literature considers *Mithraculus* as a subgenus (see Wagner, 1990). The two most popular *Mithraculus* species in the marine aquarium hobby occur in the Caribbean and are the red ruby crab *M. forceps* (A. Milne-Edwards, 1875) and the green emerald crab *M. sculptus* (Lamarck, 1818). Commonly used to control the outbursts of bubble algae *Valonia* spp C. Aghardh, 1823 in reef displays, they are also known to graze on numerous other species of algae (Coen, 1988a). The larval development of both species (two zoeae plus one megalopa) is well known from laboratory cultured specimens (Wilson *et al*., 1979; Rhyne *et al*., 2006). Apart from size, both zoeal stages can be distinguished by several morphological features: zoea I displays sessile eyes and four long plumose natatory setae in the terminal region of the exopod of the first and second maxillipeds, while zoea II exhibits mobile eyes and six long plumose natatory setae in the terminal region of the exopod of the first and second maxillipeds. The interesting results achieved by Tunberg & Creswell (1988) for the related species, Caribbean king crab *Mithrax spinosissimus* (Lamarck, 1818), recording survivals of up to 80% to first crab, prompted researchers to perform preliminary larval culture trials to evaluate the aquaculture potential of *M. forceps* and *M. sculptus*. Rhyne *et al.* (2005) verified that both species can be successfully raised to the first crab stage using newly hatched *Artemia* nauplii as larval prey. The use of enriched *Artemia* did not improve survival for both species. Although cultured larvae of *M. forceps* and *M. sculptus* reached the megalopa stage in only 5 days and displayed survivals higher than 92%, for larvae being fed *Artemia* nauplii or enriched metanauplii a significantly higher percentage of megalopa of *M. forceps* (almost 80%) was able to moult to first crab. Less than 26% of cultured *M. sculptus* megalopa were able to moult to first crab (Rhyne *et al*., 2005). The occurrence of mass mortalities of cultured crab larvae when moulting to megalopa or to first crab has been documented in several species (Harms & Seeger, 1989; Lárez *et al*., 2000; Hamasaki *et al*., 2006). Deficiencies in the fatty acid composition of larval prey have been considered to be potentially responsible for poor survival rates exhibited in the mass cultures of brachyuran larvae (Suprayudi *et al*., 2004a, b). Another aspect that may negatively affect metamorphosis to first crab is the absence of suitable settlement substrates (Forward *et al*., 2001). The specificity displayed by the post-larval stages of some decapod species for some substrates (Stevens & Kittaka, 1998; Stevens, 2003) may promote the occurrence of extended larval development when such settlement cues are absent, with developing larvae postponing metamorphosis (Forward *et al*., 1994, 1996; Wolcott & De Vries, 1994; Brumbaugh & McConaugha, 1995; Gebauer *et al*., 2003). *Mithraculus sculptus* is known to display facultative associations with

the coralline alga *Neogoniolithon strictum* (Foslie) and the branching coral *Porites porites* (Pallas, 1766) and plays a key role in the control of algal epibionts that may overgrow these organisms (Coen, 1988b; Stachowicz & Hay, 1996). Apparently, *M. sculptus* occupies a more restricted habitat than *M. forceps*. Rhyne *et al.* (2005) have hypothesized that during larval culture appropriate settlement cues could be missing for *M. sculptus* megalopa, which cannot delay metamorphosis indefinitely. This extension of larval development ends either with the death of the developing larvae or with spontaneous metamorphosis, which sometimes produces smaller sized juveniles (Gebauer *et al.*, 1999, 2003). If developing larvae concentrate all their energies in the search for a suitable habitat to settle, a significant decrease in the ingestion rate during late megalopa stage may occur. Rhyne *et al.* (2005) suggested that the reduced post-metamorphic body size recorded for cultured *M. sculptus* may be a consequence of an increased utilization of internal energetic reserves as a result of a prolonged pre-metamorphic period with little or no feeding activity.

Rhyne *et al.* (2005) suggested that when more than one marine ornamental species is sold to perform the same task, as in the case of *M. sculptus* and *M. forceps* being employed to control the growth of nuisance algae, culture efforts should focus on the less common species in the trade, namely the one achieving higher market values and consequently having less competition from the collection and trade of wild specimens. The promising results recorded in the initial culture trials of *M. forceps*, the most uncommon species in the trade, have pointed to this species as an ideal candidate for aquaculture. The study by Penha-Lopes *et al.* (2005) optimized the larval culture protocols for *M. forceps*, employing the upwelling culture system described by Calado *et al.* (2003a) to raise newly hatched zoeae of *M. forceps* to the stage of megalopa. For this specific culture period, Penha-Lopes *et al.* (2005) recommend a culture density of 40 larvae per liter, a water temperature of 28°C and the supply of seven newly hatched *Artemia* nauplii per milliliter. Cultured megalopa should then be transferred to a different rearing system based on the one designed by Tunberg & Creswell (1988) to culture *M. spinosissimus*, without changing either water temperature or larval prey density. According to Penha-Lopes *et al.* (2005), this system consists of a large and shallow recirculated water table, which is supplied with approximately 20 liters per minute of 5 μm filtered sea water. Previously cultured megalopa are placed in 30 cm diameter polyvinyl chloride (PVC) rings, which are kept suspended in the water column (Figure 9.5). Each PVC ring is gravity fed from a head tank through a 2 mm inlet positioned in the central area of the ring, at a constant flow of 0.5 liters per minute. A 200 μm mesh screen attached to the bottom of the PVC ring, combined with complete water renewal every 3 h, ensure the maintenance of good water quality and enough *Artemia* nauplii inside each PVC ring. Penha-Lopes *et al.* (2005) considered 4500 individuals per square meter as an optimal stocking density for the culture of megalopa to first crab using the PVC rings described above. Megalopa are easier to handle

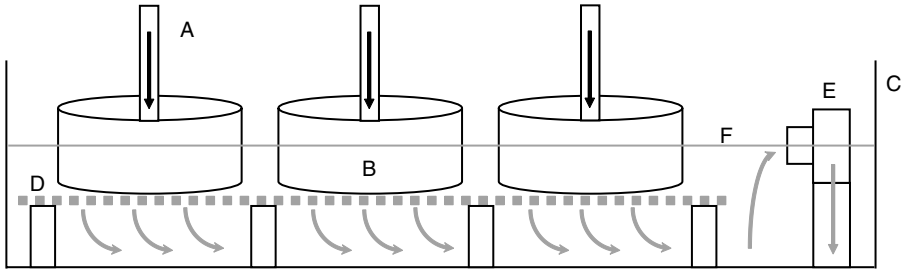


Figure 9.5 Juvenile crab grow-out system: A, inflow pipe; B, PVC rings (30 cm diameter) with 200 μm mesh bottom where crab megalopa are stocked; C, water table; D, egg crate to support PVC rings above the bottom of the water table; E, outflow pipe regulating water level; F, water level; black arrows represent inflowing water; grey arrows represent outflowing water.

than early juvenile crabs, since they cling strongly to the substrate and are less fragile. In this way, culturing megalopa in the modified version of Tunberg & Creswell's (1988) system significantly reduces juvenile *M. forceps* mortality induced by handling stress. Employing upwelling cylindrico-conical tanks during the culture of branchyuran crabs' megalopa commonly induces high mortality rates as a result of the aggregation of cultured specimens at the base of the culture vessels. Genodepa *et al.* (2004b) point out that the tangling at the bottom of culture vessels of cultured megalopa which already possess developed chelipeds appears to promote agonistic behaviors and higher rates of cannibalism. When cultured larvae moult asynchronously, it is not unusual to record the first individuals reaching the stage of megalopa cannibalizing conspecifics remaining in the zoeal stage. Additionally, the accumulation of debris (e.g. moults, dead specimens and feces) in the bottom of the tank is likely to cause stress to cultured specimens and promote infection by opportunistic pathogenic microorganisms.

When culturing juvenile crabs, the major factors that can significantly influence survival and growth rates are cannibalism (Triño *et al.*, 1999; Sainte-Marie & Lafrance, 2002), food availability and quality (Millikin *et al.*, 1980; Hartnoll, 1982; Sheen, 2000; Jee *et al.*, 2007), overcrowding stress and reduced mobility (Wilber & Wilber, 1989; Dittel *et al.*, 1995; Stachowicz & Hay, 1999; Rodriguez *et al.*, 2003) and social environment (Bliss & Boyer, 1964; Wilber & Wilber, 1991). Hartnoll & Bryant (2001) demonstrated that the effect of temperature on the intermoult increment of crabs is related to the absence (indeterminate species) or presence (determinate species) of a terminate moult. As with all majoid crabs (Hartnoll, 1982), *M. forceps* is a determinate species. Penha-Lopes *et al.* (2006a) verified that, despite the fact that higher culture temperatures (28°C) promote similar moult increments, the existence of a shorter intermoult period was responsible for a higher growth of juvenile *M. forceps*. Employing the culture system designed by Tunberg & Creswell (1988) with the modifications suggested by Penha-Lopes *et al.* (2005), the authors were

able to culture juveniles of *M. forceps* in PVC rings (750 mm in diameter and 50 mm high) communally and did not detect any cannibalistic behavior since dead specimens were intact. In general, the authors recorded a decrease in survival of juvenile crabs and an increase in intermoult period duration with increasing stocking density. At the end of the experimental grow-out period (4 weeks) crabs stocked at a density of 60 crabs per ring displayed lower survivals than those stocked at 5 and 15 crabs per ring. Penha-Lopes *et al.* (2006a) considered that the higher number of interactions between crabs cultured at higher densities (30 and 60 crabs per ring) might have induced the existence of physiological stress and eventually contributed to the lower growth rate and survival recorded at higher culture densities. The authors recommend using a stocking density of 15 crabs per ring (approximately 3400 crabs per square meter) if survival and growth rates are the single evaluation criterion for optimal culture conditions. None the less, in order to maximize production efforts, higher rearing densities can certainly be used. Considering nutrition, juvenile crabs fed on newly hatched *Artemia* nauplii and microalgae (*Amphora* spp.) displayed the highest growth rates (Penha-Lopes *et al.*, 2006a). However, cultured juveniles also readily ingest inert particles, namely frozen newly hatched *Artemia* nauplii, as well as penaeid shrimp grow-out pellets. These results provide valuable indications for a future formulation of an artificial feed that can fulfil the nutritional requirements of these omnivorous crabs and ensure that cultured juveniles display the attractive coloration of wild specimens.

Although ovigerous *M. forceps* females are available in the wild throughout the year (Mantellato *et al.*, 2003), thus ensuring a regular supply of larvae for captive culture, reducing the number of broodstock required for hatchery production cuts production costs and promotes the conservation of these highly valued resources. In an effort to decrease the need to collect ovigerous females, Penha-Lopes *et al.* (2006b) have shown that females kept in captivity could produce a second clutch of viable embryos using stored sperm from previous male insemination(s). The capacity to use stored sperm to produce a new clutch of embryos has been observed in other crustacean species, often displaying poor fertility or larval viability (Diesel, 1988; Paul & Paul, 1992; Sainte-Marie & Carriere, 1995). However, Penha-Lopes *et al.* (2006b) did not record the existence of unfertilized eggs or deformed larvae, with the only 'negative'aspect detected being the smaller size of newly settled juveniles cultured from the second clutch of embryos. The authors suggest that these results may be due to the fact that larvae produced from a second clutch receive less energy from the parental female and that the grow-out performance of these juveniles still needs to be investigated.

In comparison to other marine ornamental decapods, crabs of the genus *Mithraculus* display the remarkable advantage of perfectly enduring communal shipping, which significantly reduces their transportation cost (Calado,

unpubl. obs.). This particular aspect may be crucial for a commercial enterprise when deciding which marine ornamental decapod species to produce in captivity.

As previously mentioned, crabs from the genus *Percnon*, commonly known as Sally Lightfoot or spray crabs, are also facing increasing demand in the marine aquarium trade as a consequence of their ability to control the growth of nuisance algae (Sprung, 2001, 2002). The most popular species is *Percnon gibbesi* (H. Milne Edwards, 1853), since this member of the family Plagusiidae Dana, 1851 does not venture out of the water like other grapsoid crabs and consequently will not crawl out of reef displays. Although it is sometimes stated that large specimens may be a threat to other invertebrates and small fishes in marine aquariums (Sprung, 2001; Nilsen & Fosså, 2002; Shimek, 2004), a study on the feeding habits of wild populations by Puccio *et al.* (2006) revealed that *P. gibbesi* is a strictly herbivorous species. This conclusion was based on the morphological features displayed by the chelipeds of *P. gibbesi,* on the feeding adaptations exhibited by its gastric mill, and through the analysis of its stomach contents. The largest challenge for the culture of this species in captivity will certainly be large-scale larval culture. The zoeal stages of grapsoid crabs are known to be among the most difficult brachyuran larvae to culture in captivity. Such difficulties are reflected in the reduced number of complete larval series described from laboratory-cultured material (Cuesta & Rodríguez, 2000). It displays a relatively long larval period, with a total of six zoeal stages plus one megalopa (Paula & Hartnoll, 1989). Zoeal stages are characterized by the presence of an extremely long and slender rostrum, as well as large carapace spines, which assume remarkable proportions in later stages (Figure 9.4). During early zoeal culture, microalgae, as well as rotifers and/or copepodites will probably be more adequate larval prey than newly hatched *Artemia*. This requirement may discourage commercial enterprises from choosing commercial-scale production of *Percnon*, particularly owing to the less demanding culture requirements of larval *Mithraculus*, whose first zoeal stage readily accepts newly hatched *Artemia* nauplii. However, since the large-sized megalopa of *Percnon* metamorphoses to a relatively large first crab (Hartnoll, 1992), the juvenile grow-out period may be of short duration, with juveniles reaching marketable sizes a few weeks after settlement. In the aquarium trade, smaller-sized *Percnon* are commonly preferred over large individuals and usually reach higher prices than *Mithraculus*. The gregarious nature of *Percnon*, which occurs in the wild in dense populations (Deudero *et al*., 2005), is a good indicator of the possibility of employing communal rearing during juvenile grow-out. Additionally, the fact that *P. gibbesi* is a strictly herbivorous species may facilitate the formulation of an artificial grow-out diet using less expensive ingredients (e.g. Sudaryono *et al*., 1999; Davis *et al*., 2002; Patnaik *et al*., 2006). Curiously, *P. gibbesi* is now an invasive species in the Mediterranean Sea (Relini *et al*., 2000; Pipitone *et al*., 2001), which is rapidly

spreading and establishing large populations (Deudero *et al*., 2005; Thessalou-Legaki *et al*., 2006; Yokes & Galil, 2006). Whether this invasion resulted from *Percnon* larvae entering the Mediterranean through Atlantic currents, as a consequence of natural dispersal (Abelló *et al*., 2003), or was introduced as a result of crabs being transported by recreational and/or fishing vessels (Cannicci *et al*., 2006), remains an unanswered question. An interesting approach to control this invasive species would be to promote its collection and trade for marine aquariums. Apart from becoming a profitable activity as a result of the high prices that this species achieves in European markets, this strategic exploration would certainly place pressure on the wild populations of *P. gibbesi*, which continue to proliferate in the Mediterranean.

Crabs in the genus *Lybia* H. Milne Edwards, 1834, belong to the family Xanthidae MacLeay, 1838, and are commonly known in the hobby as pom-pom crabs. These are probably the crabs that command the highest market values in the aquarium trade. Their popular name reflects the amazing association displayed by these crabs and the sea anemones *Triactis producta* Klunzinger, 1877. *Lybia* specimens carry an anemone in each cheliped, and their appendages are equipped with numerous spines to keep the anemone in place. These crabs readily employ their anemones for defense as well as to gather food.

Currently, there is no information available on how ovigerous *Lybia* females position themselves during larval release and if newly hatched larvae are somehow vulnerable to the stings of the anemones carried by the parental crab. When holding a reproductive pair in a maturation tank, this aspect is even more relevant, since newly hatched larvae may also be vulnerable to the anemones carried by the male crab. If these organisms do not display any particular adaptation to prevent their partner anemones from capturing newly hatched larvae, maturation systems designed for this particular species should optimize larval collection and ensure that water flow does not retain the larvae in the parental area for an extended period.

Xanthid crabs do not display a particularly long larval development, usually exhibiting four zoeal stages plus one megalopa (González-Gordillo *et al*., 2001). The different zoeal stages may be distinguished by several morphological features, although the most simple to observe is probably the number of long terminal plumose natatory setae present in the distal segment of the exopod of the first and second maxillipeds: four during zoea I, six during zoea II, eight during zoea III and ten during zoea IV (e.g. Paula & dos Santos, 2000; Ko & Clark, 2002). Xanthid larvae are commonly raised in captivity to provide laboratory-cultured material for larval descriptions using simple culture methodologies (Ingle & Clark, 1977). The system described by Calado *et al*. (2003a) with the modifications proposed by Penha-Lopes *et al*. (2005) may allow a large number of *Lybia* larvae to be cultured using newly hatched *Artemia* nauplii. Nevertheless, supplying rotifers to the first zoeal stage may significantly increase survival until metamorphosis. So far, no study has ever

examined whether or not the partner anemones *T. producta* play any role during the metamorphosis of *Lybia* megalopa. If the association with these anemones occurs during an early post-settlement stage, it is possible that *Lybia* megalopa will prefer to settle near these organisms.

Karplus *et al*. (1998) studied the fighting behavior of *Lybia edmonsoni* Takeda and Miyake, 1970, and revealed the occurrence of frequent agonistic interactions between conspecifics. However, fighting individuals only rarely, or incidentally, touched their opponents with the anemones. The authors formulated three potential explanations for such fighting behavior:

(1) the anemones are highly toxic to *Lybia*, which could severely damage the winner and loser of the fights;
(2) the anemones are not toxic to *Lybia* and are an insufficient weapon; and
(3) the anemones are a valuable resource, with fighting crabs not risking their loss or damage.

Male and female *Lybia* do not display sexual dimorphism concerning the size of their chelae, as recorded for other decapods (Hyatt, 1983), and therefore have similar areas for holding anemones. This aspect may be responsible for the similar fighting behaviors displayed by both genders.

Despite exhibiting conspicuous fighting behavior, *Lybia* species may be a suitable candidate for communal rearing of juvenile specimens. The main reason for this assumption is their highly ritualized fighting behavior, which minimizes contacts between opponents (Karplus *et al*., 1998). Although these crabs have always been recorded in the wild carrying anemones in their chelipeds (Duerden, 1905), specimens kept in captivity without anemones are able to survive for several months (Karplus *et al*., 1998). This aspect may be important during grow-out, since it may be possible to raise juvenile specimens without providing them with their partner anemones. However, any commercial enterprise willing to culture these organisms will need to run a parallel culture of the anemone *T. producta* in order to allow juvenile crabs reaching commercial size to be sold carrying their trademark 'pom-pom'. The behavior displayed by *L. edmonsoni* in captivity, forcing asexual reproduction upon their anemones by splitting them into smaller individuals (Karplus *et al*., 1998), may be an interesting way of ensuring that enough anemones are always available in captivity.

Coral crabs are also xanthoid crabs but are members of two distinct families: Trapeziidae Miers, 1886, and Tetraliidae Castro, Ng and Ahyong, 2004. These crabs are commonly present in the trade as 'bonus' creatures that are not sold individually but instead commonly enter reef aquariums 'hiding' among the branches of hard coral colonies. While coral crabs of genus *Trapezia* Latreille, 1825 commonly occur in corals of genus *Pocillopora* Lamarck, 1816, those in the genus *Tetralia* Dana, 1851 are known to mainly occupy stony corals of genus *Acropora* Oken, 1815. Morphologically, both genera can be distinguished by

the anterior region of their carapace, since the genus *Trapezia* exhibits five wide lobes and the genus *Tetralia* displays a large number of minute teeth, making the lobes in the anterior region of the carapace hardly discernable. The two genera may also be distinguished by their chelipeds, with the species belonging to genus *Tetralia* displaying these appendages with conspicuously dissimilar sizes (Castro *et al*., 2004). Given the extreme morphological similarity between some coral crab species, Odinetz (1984) highlights the importance of using live specimens' coloration for accurate species identification.

These crabs are obligate symbionts, feeding from coral mucus and entrapped particulate material, as well as gaining protection from predators when on their coral host (Knudsen, 1967; Patton, 1974; Vytopil & Willis, 2001). In the wild these organisms are also known to play an important role in the protection of their host coral against predation by the crown of thorns sea star *Acanthaster planci* (Linnaeus, 1758), with larger sized *Trapezia* spp being more effective than smaller *Tetralia* spp (Pratchett, 2001). Apparently, whereas *Trapezia* spp commonly attack the thorns of the starfish, breaking them off at the pedicel, *Tetralia* spp mainly pinch at the tube feet and do not seem to cause any serious injury to the predatory starfish (Glynn, 1982; Pratchett *et al*., 2000). Regardless of the coral colony size, adults of these species nearly always occur as heterosexual pairs, with a single pair of a given species per colony, which they actively defend against conspecifics (Castro, 1976). However, whereas coral crabs vigorously defend their hosts against adult conspecifics of the same sex, the presence of juveniles is commonly tolerated (Huber, 1983). The number of eggs produced is highly correlated with the size of female coral crabs (Preston, 1971; Gotelli *et al*., 1985). However, since crab size and colony size are poorly correlated, the correlation between crab size and clutch size should be reduced if clutch size changes with the dimension of the host coral colony. Therefore, according to Huber & Coles (1986), the number of eggs produced per clutch is probably not a strong function of colony size. Females are usually ovigerous year round and the development time of an embryo clutch is about 1 month, with the interval between larval hatching and the extrusion of a new clutch being only 1–2 days (Huber & Coles, 1986). From a commercial perspective, given the peculiar utilization of their coral host colony, it could be advantageous to select a large-sized coral colony harbouring reproductive pairs from different species of coral crabs. None the less, this approach would only maximize the use of available room in maturation systems if the size of the tank required to hold large coral colonies is not significantly superior to that of smaller sized tanks that may hold a single reproductive coral crab pair in a smaller sized colony. Additionally, the economic investment required to buy large coral colonies, as well as the negative aspects associated with the harvest of large specimens from the wild, may make this alternative less appealing. Obviously, maturation systems holding hard coral colonies must be equipped with suitable illumination (e.g. metal halide or T5 lamps) and ensure low nutrient levels to fulfil

the necessary requirements for the successful husbandry of these organisms (Sprung, 1999).

As stated previously, xanthoid crabs display four zoeal stages plus one megalopa during their larval development. Xanthoid larval stages have been successfully cultured in captivity using newly hatched *Artemia* nauplii as larval preys, although the addition of rotifers, at least during the first zoeal stage, will certainly improve survival. Although adult coral crabs associate with coral colonies, very little is known about their larval development and settlement. Given the significant number of post-megalopa *Trapezia* juveniles recorded by Gotelli *et al*. (1985) in the colonies of *Pocillopora*, it is reasonable to assume that chemical cues released by the host coral species and/or conspecifics may induce the metamorphosis of developing megalopa. It is also possible that the megalopa of coral crabs do not require any particular cue to induce metamorphosis and after settlement migrate to a coral colony occupied by conspecifics.

Whereas most of these organisms commonly exhibit strong intraspecific territoriality, their interspecific territoriality is substantially reduced. However, some exceptions to this rule may occur, with Huber (1987) reporting interspecific aggression between two species of *Trapezia*. Nevertheless, the coexistence of competing species in the same coral colony is commonly recorded, even if overlapping resource utilization may occur (Huber & Coles, 1986). These aspects of coral crab biology may be used to develop a multi-species grow-out approach in which different species of coral crabs may share the same coral colony. However, the commercial-scale production of coral crabs may only be possible if enough hard coral colonies are regularly available during grow-out. Given the increasing interest of enterprises in the captive propagation of hard branching corals in captivity (Calfo, 2007), it may be a wise commercial approach to couple coral propagation efforts with production of coral crabs. In fact, such a strategy may significantly increase the survival of cultured colonies, since those with symbiotic crabs shed substantially more sediments which are commonly deposited on coral surfaces (Stewart *et al*., 2006). Additionally, coral crabs appear to be more effective at removing sediments with grain sizes that are most damaging to coral tissues.

9.4 Marine ornamental lobsters

In the marine aquarium trade a wide group of taxonomically distinct decapods are traditionally sold as lobsters (Table 9.1). These organisms range from true spiny lobsters belonging to the infraorder Palinura Latreille, 1802, and the family Palinuridae Latreille, 1802, to dwarf reef lobsters, which belong to the infraorder Astacidea Latreille, 1802 and are members of the family Enoplometopidae de Saint Laurent, 1988 (Figure 9.2).

The most commonly traded spiny lobsters in the marine aquarium industry are the blue spiny lobster *Panulirus versicolor* (Latreille, 1804) and the long-handed spiny lobster *Justitia longimanus* (H. Milne Edwards, 1837). Given the well-known bottlenecks that still impair the commercial culture of spiny lobsters for human consumption (Kittaka, 2000), it is highly unlikely that marine ornamental spiny lobsters will be the target of culture efforts in the near future. However, *P. versicolor* is also targeted for human consumption, and puerulus collection has become a solid alternative to larval spiny lobster culture (Mills & Crear, 2004). Since hobbyists prefer to keep small-sized juveniles in reef displays (Shimek, 2004), growing newly settled *P. versicolor* to just a few centimeters and selling them to the marine aquarium industry may be an interesting parallel activity for enterprises raising spiny lobsters for human consumption. The long-handed spiny lobster *J. longimanus* belongs to a group of spiny lobsters that have 'false claws' and also display extended larval development (Figure 9.6) (Robertson, 1969; Johnson & Robertson, 1970). Given the similarities recorded by Baisre (1969) between the larvae of *Justitia* Holthuis, 1946 and those of *Panulirus* White, 1847, it is likely that the culture of their phyllosoma larvae will also present the same bottlenecks recorded for other spiny lobster genera. However, it is possible that *J. longimanus* may become a valuable by-catch of wild puerulus collection for captive grow-out, which will certainly not be overlooked by enterprises.

Dwarf reef lobsters belong to a puzzling group of decapods whose systematic affinities are still being discussed and which are apparently not as close to other clawed lobsters as once thought (Martin & Davis, 2001; Ahyong & O'Meally, 2004). The most commonly imported species are the violet or Daum's reef lobster *Enoplometopus daumi* Holthuis, 1983, Debelius's reef lobster *E. debelius* Holthuis, 1983, and the red reef lobster *E. occidentalis* (J. W. Randall, 1840), while the Atlantic reef lobster *E. antillensis* Lütken, 1865 is more rarely available. These organisms occur alone or as mated pairs in the wild and are known to display highly aggressive behavior towards same-sex conspecifics (Cushing & Reese, 1988). These authors reported that the fights between members of the same gender commonly resulted in more or less severe damage and that even when one of the specimens retreated the winner continued to pursue the loser, which seemed to indicate a lack of submissive behavior. Additionally, it was also reported that cheliped-to-cheliped contact appears to be obligate for sexual recognition among *Enoplometopus* specimens. At least when pairing *E. daumi*, *E. debelius* and *E. occidentalis* it is important to always place the male in a tank already stocked with a female (see Figure 9.3 for the diagnosis of sexual features), since the opposite will always trigger strong agonistic behaviors, with the female ablating the male chelipeds (G. Penha-Lopes, pers. comm.). According to Debelius (2001), females may also try to chase away males after egg extrusion and fertilization. This behavior may require mated pairs to be stocked in large maturation tanks (at least 60 cm in length) with several shelters, in order to allow the male specimen to

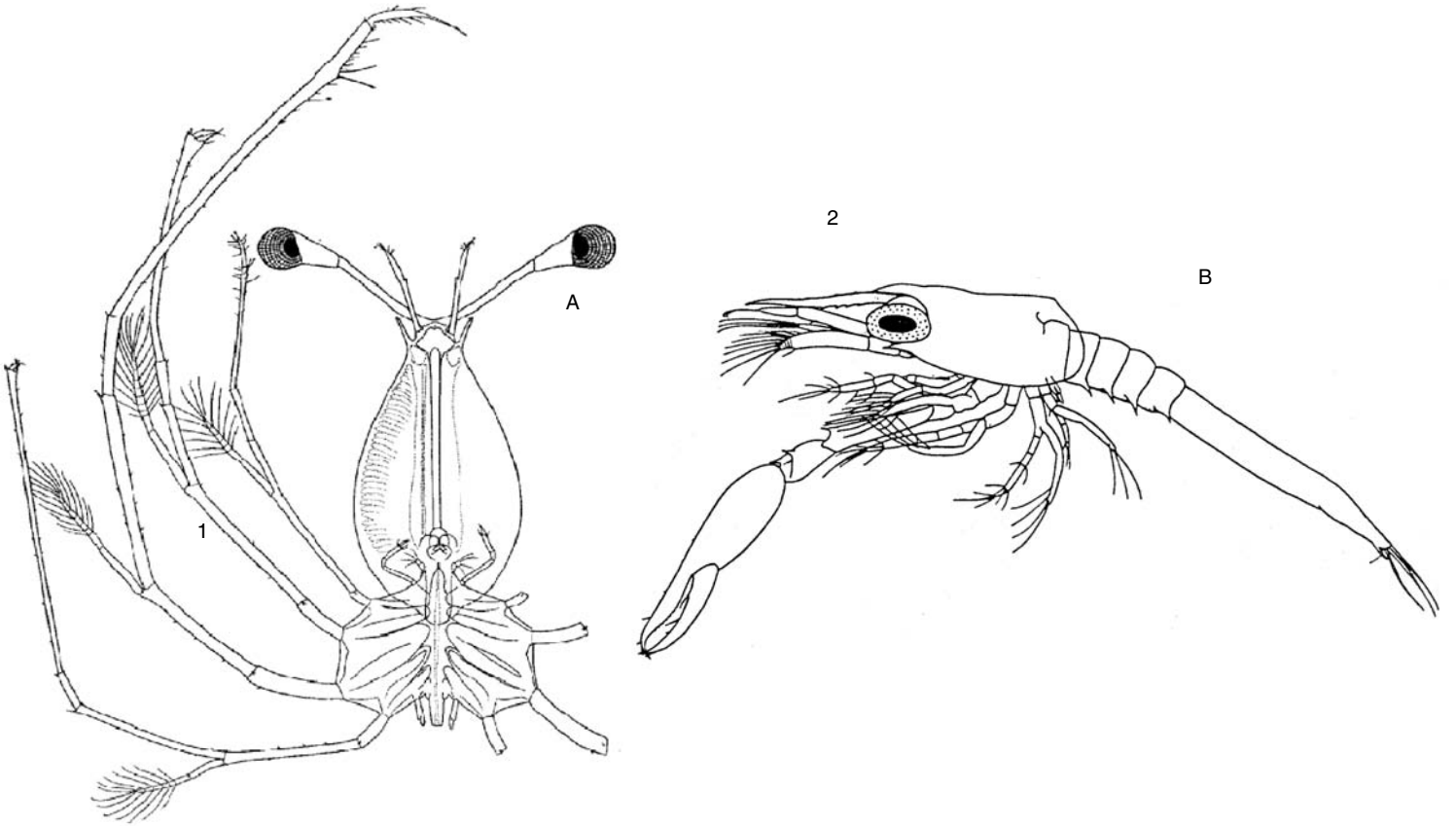


Figure 9.6 'Typical' *Justitia* phyllosoma [the equivalent to other decapod zoeae, (A)] and enoplometopid zoea (B); commonly damaged larval appendages during culture: pereiopods (1) and rostrum (2) (larvae not to scale) [adapted from Robertson (1969) and Gurney (1938b)].

safely retreat if threatened by the female. The average time between spawns of *E. daumi* and *E. debelius* kept in captivity at 28°C is approximately 25 and 35 days, respectively (G. Penha-Lopes, pers. comm.). Currently, there is still a great lack of knowledge of the morphology of *Enoplometopus* larvae, as well as of their average larval development period. *Enoplometopus debelius* displays at least seven zoeal stages and metamorphosis occurs about 45 days post-hatching (G. Penha-Lopes, pers. comm.). Newly hatched larvae of at least some *Enoplometopus* species (e.g. *E. debelius*) already display large chelae (Figure 9.6) (Debelius, 2001; Calado, unpubl. obs.). Newly hatched zoeae may readily capture *Artemia* nauplii, or even enriched metanauplii, while late zoeal stages are known to be able to capture and ingest large live prey such as newly hatched *Lysmata* larvae (A.L. Rhyne, pers. comm.). *Enoplometopus* larval rearing at high stocking densities, particularly during late zoeal stages, promotes the occurrence of cannibalism. Similar situations have already been recorded during the larval culture of other clawed lobsters, namely *Homarus* spp (Sastry & Zeitlin-Hale, 1977; Nicosia & Lavalli, 1999; Jørstad *et al*., 2005). Newly settled *E. debelius* display a total length of approximately 18 mm (G. Penha-Lopes, pers. comm.). During early post-settlement stages, communal rearing of *Enoplometopus* may be possible, since these organisms must have only a small minimum shelter area. As long as enough shelters are present, agonistic interactions between conspecifics may be reduced. This approach has already been tested by Boudreau *et al.* (1990) using newly settled clawed lobster *Homarus*, with shelter opacity being a more important factor for shelter selection than shelter shape or entrance format. The authors also report the co-occurrence of four small juveniles in a 40 cm^2 shelter, suggesting that young lobsters must achieve a balance between the risks of intraspecific agonistic behavior when sharing a shelter and the risks of being exposed to predation. However, there is always the risk of juvenile *Enoplometopus* only reaching marketable sizes after requiring larger shelter areas and starting to exhibit stronger agonistic responses towards conspecifics. In this situation, an individual grow-out approach similar to the ones used for *Homarus* (e.g. Mickelsen *et al*., 1978) and described in the section addressing the culture of juvenile boxer shrimps *Stenopus* must be employed.

Chapter 10

Future Improvements in Marine Ornamental Shrimp Culture

10.1 Broodstock management and maturation

Because of the constraints recorded during early culture trials of marine ornamental shrimps, most research efforts have focused on the development of commercial-scale larval rearing protocols for the most valuable species (*Lysmata* and *Stenopus*). The relative simplicity of triggering maturation of marine ornamental shrimps in captivity (e.g. there is no need to perform eyestalk ablation: Zhang *et al.*, 1997a) has led to the erroneous belief that broodstock diets were not an issue for the successful culture of marine ornamental shrimps. However, the superior results commonly achieved in larval culture trials when using larvae produced by shrimps carrying embryos spawned in the wild (e.g. Calado *et al.*, 2005a), in opposition to larvae produced by shrimps kept in captivity over several weeks or months, have clearly demonstrated the importance of broodstock nutrition. It is possible that some of the problems usually arising during the culture of larvae produced by shrimps that have spawned in captivity may be a direct consequence of deficient broodstock nutrition. The lack of studies addressing the feeding regimes of marine ornamental shrimps in the wild limits current knowledge on the nutritional requirements of these organisms, especially during maturation. However, shrimps display digestive tracts with complex mechanisms for triturating and separating fine food particles, such as the gastric mill (Jha & Homechaudhuri, 2001), which make it extremely difficult to identify ingested dietary food items without hard parts. Additionally, due to the peculiar feeding habits of some marine ornamental shrimps (e.g. cleaner shrimps ingest fish ectoparasites, though not exclusively, and harlequin shrimps feed on starfishes), it may be difficult to understand their dietary requirements in captivity.

Instead of relying exclusively on fresh and frozen dietary items to feed reproductive broodstock, the use of suitable artificial diets in the short term

would be a major breakthrough for aquaculture of marine ornamental shrimps. Developing a balanced artificial diet that can fulfil the nutritional needs of broodstock from the main cultured species during maturation will not be an easy task, mainly because of the phylogenetic distance of marine ornamental shrimp families. In fact, it will not be surprising if species-specific requirements are also detected. The biochemical analysis of newly extruded eggs would be an excellent starting point for the formulation of artificial diets, since their composition will reflect the major nutrients being mobilized by female shrimps during maturation. This analysis would also provide some insight into the similarity of the nutritional requirements displayed by different marine ornamental shrimp species. Despite the difficulties that need to be overcome, employing an artificial diet, at least to partially feed reproductive broodstock, has the advantage of displaying a stable and more easily adapted nutritional composition. Simultaneously, it would also minimize the risks of contamination of maturation systems and of exposing broodstock and newly hatched larvae to potential pathogens.

When using fresh or frozen foods, broodstock shrimps are not generally fed during the night period. Curiously, this is also the most active period for most marine ornamental species. This may explain why shrimps so eagerly accept the first meal provided early in the morning, even when less appealing dietary items are offered. Apart from the nutritional issues that these prolonged periods of involuntary starvation may promote, starved broodstock shrimps will more readily cannibalize newly hatched larvae, since these are commonly released during the night time (Simões *et al.*, 2002). By using an artificial diet, shrimps may also be fed during the night period through automatic feeding devices, eliminating the deleterious effects of starvation.

After the development of suitable culture protocols, the next step will probably be to start selective breeding program for marine ornamental shrimps, a strategy implemented in penaeid shrimp culture (Lester, 1983; Ibarra *et al.*, 2007) and that has already provided some remarkable advances in growth performance and disease resistance (e.g. Argue *et al.*, 2002; Preston *et al.*, 2004). As pointed out by Li *et al.* (2006) for selective breeding programs for penaeid shrimp populations, with increasing selection period, genetic diversity will tend to be reduced and consecutive generations will become progressively less differentiated. This approach may allow the positive selection of fast-growing shrimps and/or unusual coloration patterns.

One problem that must be faced when undergoing selective breeding programmes is inbreeding. Keys *et al.* (2004) have shown that inbreeding commonly has a negative impact on the growth performance of cultured shrimps but not on their survival. Moss *et al.* (2007) suggested that moderate to high levels of inbreeding (>10%) should be avoided in shrimp breeding programmes, particularly if shrimps may face stressful conditions during grow-out.

10.2 Larval culture

This field is certainly the one that has deserved more attention from researchers working on marine ornamental shrimp aquaculture. The initial optimism of researchers working on the larval culture of these organisms highlighted how the technology available for penaeid shrimp larval culture would allow the bottlenecks identified in preliminary trials to be easily overcome (Fletcher *et al*., 1995). However, this optimism was rapidly replaced by apprehension when later studies revealed the difficulty of the task. Penaeid shrimps hatch as nauplii and require microalgae during their early culture. Marine ornamental shrimps hatch as a more advanced larval stage (zoea), which is already able to capture larger sized dietary items.

The naturally extended larval development of most marine ornamental shrimp species, commonly displaying eight or more zoeal stages plus one megalopa, coupled with their remarkable ability to delay metamorphosis through mark-time moulting (Calado *et al*., 2003b), is the major bottleneck impeding the commercial-scale production of these organisms. The occurrence of extended larval development in cultured marine ornamental shrimps was commonly explained by the absence of suitable settlement cues, which would be responsible for triggering the mechanisms associated with metamorphosis (Fletcher *et al*., 1995). However, current evidence supports a different viewpoint, namely that unsuitable larval nutrition may lead developing larvae to delay their development (Calado *et al*., 2001a, 2005a). Apparently, cultured larvae will only advance in zoeal development and eventually metamorphose after building up enough energetic reserves in the previous larval stage (Calado *et al*., 2007b). Curiously, this hypothesis had already been addressed several decades ago by Gurney & Lebour (1941) to explain the existence of 'giant' decapod larvae in the plankton. In this way, since developing marine ornamental shrimps more than double (some may even triple) their body size during larval development (e.g. Calado *et al*., 2004), they undergo a gradual shift in their dietary prey preference. While newly hatched larvae of some species readily prey on *Artemia* nauplii and metanauplii, others, sometimes even in the same genus (e.g. *Lysmata*), require smaller prey (e.g. rotifers, copepods and copepodites). The use of microalgae also appears to play an important nutritional role, at least during the early zoeal stages of some ornamental shrimps (Simões *et al*., 2002). On the other hand, larger zoeal stages may even prey on live adult *Artemia*. Employing a mesocosm approach may be a viable alternative to marine ornamental shrimp culture, providing a wider scope of prey items and fulfilling the dietary needs of cultured larvae from hatching until settlement. This approach has allowed the successful culture of numerous fish and shrimp species (Palmer *et al*., 2007). Through the regular addition of microalgae with high nutritional value (with an emphasis on highly unsaturated fatty acids, namely DHA), rotifers, copepods and *Artemia*

inoculated in the rearing tank will have enough food available to complete their life cycles and establish stable populations. After an initial period, these organisms will be able to supply cultured larvae with a wide scope of potential dietary prey: rotifers, copepod nauplii, copepodites, adult copepods, newly hatched *Artemia* nauplii, metanauplii and adult *Artemia*. Apart from allowing the reproduction of dietary prey inside the culture tank, microalgae will also improve their nutritional profile, can be ingested by cultured larvae and ultimately act as probiotics against the potential action of pathogenic agents.

A more challenging task will be the culture of marine ornamental shrimp larvae in field-deployed enclosures. This approach relies on natural planktonic prey to fulfil the dietary needs of cultured larvae and has already been successfully employed to culture the zoeal stages of the Atlantic mud crab *Panopeus herbstii* H. Milne Edwards, 1834, by Epifanio *et al.* (1991, 1994). The authors employed a modified version of the enclosures designed by de Lafontaine & Leggett (1987), displaying a total volume of almost 1.5 m^3, with a 1 m diameter, 2 m deep cylinder and a 1 m deep cone attached to the bottom of the cylinder. The cylindrical portion of the enclosures displays a mesh size of 25 μm, and the mesh size in the conical end measures 53 μm. A PVC cod-end is secured to the bottom of the conical mesh to recover any samples required and 1 m diameter steel rings are placed at 1 m intervals on the exterior wall of the cylinder to keep the desired structure shape. About 0.5 m of the enclosure must remain outside the water, being attached to a floater to prevent cultured larvae from escaping from the enclosure and unwanted organisms from entering it. After stocking newly hatched larvae at the desired density, the enclosures must be inoculated with natural plankton, which is previously filtered to remove predatory organisms. According to Epifanio *et al.* (1994), the survival results displayed by larvae cultured in these enclosures were significantly higher than those displayed by larvae cultured in captivity. Larvae cultured in captivity also developed more slowly than those raised in field-deployed enclosures. However, the present methodology may only be employed when culturing autochthonous species, since there is always the risk of exotic specimens somehow escaping the enclosures. Additionally, water temperature in temperate areas may not be suitable for raising tropical species, at least throughout the year. In collection areas in Southeast Asia, this culture approach may become a valuable and more sustainable income source for local populations. The technical requirements to run maturation systems in these areas are much less complex than those required to run larval culture facilities. Local inhabitants may be easily trained to regularly capture ovigerous females, collect their newly hatched larvae in inland maturation systems, stock them in field-deployed enclosures and return the spent females to the reef. This 'low-tech' approach may provide local populations with a viable alternative to the harvest of wild marine ornamental shrimps. Additionally, it can minimize the concerns of local populations about the setup of farming facilities in western countries and the eventual decrease in

the economic benefits generated through marine ornamental species exports (Tlusty, 2002).

As for broodstock management, the development of artificial diets must also be considered as a short-term priority for ornamental shrimp larval culture. This breakthrough has already been achieved for other shrimps cultured in captivity (see, for example, Sangha *et al.*, 2000; Robinson *et al.*, 2005), which may facilitate the research required in this particular field. Apart from decreasing production costs and allowing a higher degree of automation, artificial larval diets display a more reliable nutritional profile than live diets. Marine ornamental shrimps are known to accept inert particles in early (Calado *et al.*, 2001a) and late (Palmtag & Holt, 2007) zoeal stages, and preliminary trials using inert diets have highlighted their potential (Rhyne & Lin, 2004). Artificial diets must be attractive to larvae and simultaneously supply all required nutrients during larval development. This last aspect will probably require artificial diets to be adjusted in size, texture and composition to meet the requirements of different zoeal stages. Even if complete replacement of live prey for inert diets is not possible for all species, it will be important to experimentally determine the best timing to wean marine ornamental shrimp larvae.

Studies addressing ontogenic changes in digestive enzymatic activity during the larval period will be a valuable contribution to the development of highly digestible artificial diets as well as the adjustment of species-specific requirements concerning the digestibility of diet ingredients. The biochemical analysis of yolk reserves of late-stage embryos of marine ornamental shrimps produced in the wild may provide researchers with an estimate of the main nutritional requirements that will be displayed by newly hatched larvae. The rationale for this assumption is that newly hatched larvae rapidly catabolize their remaining yolk reserves to fulfil their energetic requirements. This type of information may allow a significant reduction in mortality of early zoeal stages by mimicking the nutritional profile of dietary items with that of late-stage embryos. Identifying the nutritional requirements of late-stage larvae will certainly be a more challenging task. Only through intense plankton sampling would enough biological material be available to allow such studies to be performed. Additionally, specimens collected from the plankton would also provide valuable information on the microorganisms associated with late zoeal stages, which probably have a probiotic action. After laboratory isolation, identification and production, these microorganisms could be inoculated in intensive larval culture systems and prevent the action of opportunistic pathogens that commonly induce mortality in late zoeal stages.

10.3 Grow-out

The grow-out of juvenile marine ornamental shrimps will probably only be addressed in greater detail after the current bottlenecks affecting broodstock

maturation and larval culture have been solved. None the less, three main topics are already the subject of current research efforts:

(1) development of an artificial diet;
(2) elimination of cannibalism during grow-out; and
(3) impairment of precocious maturation of cultured specimens.

The advantages of employing artificial diets have already been extensively addressed. However, from a commercial perspective, the economic impact of not employing a formulated feed will always be higher during grow-out than during maturation or larval culture. Cultured specimens must have a reasonable market price in order to compete in the aquarium trade with less expensive shrimps collected from the wild. An obvious way to decrease production costs will be to replace the more expensive diets of fresh and frozen items by formulated ones using inexpensive and environmentally friendly ingredients. Additionally, the promising results achieved in experimental grow-out trials using newly settled marine ornamental shrimps collected from the wild (Hair *et al*., 2004; Lecaillon, 2004) are stimulating the development of suitable inexpensive artificial grow-out diets. This achievement will certainly help local populations in Southeast Asia employing this culture method to maximize their productivity as well as profitability. Again, the biochemical profile of wild juvenile specimens may prove to be an excellent starting point for the formulation of suitable grow-out diets (Calado *et al*., 2005e).

Maximizing the number of available shelters per cultured shrimp may help to reduce cannibalism but will certainly not solve this major constraint during grow-out. Cannibalism may be more easily eliminated if an artificial diet is used to grow marine ornamental shrimps. Currently, grow-out protocols rely entirely on fresh and/or frozen dietary items, and commonly do not provide food during the night period (when employers are no longer present at the facility). Unfortunately, it is during the night that most marine ornamental shrimps display higher activity and can be commonly observed foraging for food. Obviously, even if not too extended, this period of starvation will certainly promote the occurrence of cannibalism among cultured specimens. However, artificial diets may be readily supplied during a 24-hour cycle through automatic feeders. The period between meals can be optimized to reduce food waste, as well as the consequent decrease in water quality, promoting a regular supply of high quality food that may be automatically increased during periods of higher activity. A detailed knowledge of shrimps' activity patterns in captivity is vital to maximize production (Pontes *et al*., 2006). If cultured shrimps are permanently satiated, then there will be a significant decrease in the chances of these organisms cannibalizing conspecifics. Although keeping a regular amount of live prey (e.g. newly hatched nauplii or adult *Artemia*) inside grow-out tanks may significantly decrease cannibalism, this is an expensive option that increases the risks of disease introduction

and it may only be viable in relatively small-scale facilities. Cannibalism may also be significantly reduced by ensuring that specimens being grown to commercial size display similar sizes. The existence of heterogeneous individual growth is usually associated with higher rates of cannibalism (Ranjeet & Kurup, 2002). Although grading growing juveniles is a time-consuming task, it is possible that current technology employed to sort ornamental fish by computer vision (Zion *et al*., 2000) may also be adapted to marine ornamental shrimps. Karplus *et al*. (2003, 2005) highlighted how strong phototactic responses displayed by fishes are a valuable feature to implement sorting by computer vision. Since most marine ornamental shrimps exhibit strong avoidance behavior in relation to intense light sources, it is possible that this technology may be successfully adapted to these organisms.

The precocious maturation of marine ornamental juveniles was first recorded by Calado *et al*. (2005e) during the grow-out of Monaco shrimps *Lysmata seticaudata*. Several cultured specimens were recorded shifting from male to simultaneous hermaphrodite phase with only 15 mm of total body length and some of them were even observed already incubating fertilized embryos. The detection of this event was only possible as a consequence of the existence of population biology studies describing the size at the onset of sexual maturation for the female portion of the *L. seticaudata* sexual system. According to Dohrn (1950) and Carlisle (1953), ovigerous wild Monaco shrimps have only been recorded after reaching at least 30 mm in total length. Unfortunately, biological studies on the onset of sexual maturity are not available for most ornamental shrimp species, which may allow precocious maturation to go unnoticed during grow-out. From a commercial perspective, the major drawback in the occurrence of precocious maturation in juvenile female or simultaneous hermaphrodite shrimps during grow-out is the reallocation of a large portion of energetic reserves from growth to the production of energetically expensive vitellogenic oocytes. This event slows down growth rates and increases the culture period required to grow juvenile shrimps to a marketable size. Obviously, this aspect will be reflected in an increase in production costs.

Calado & Dinis (2007) were able to minimize the precocious maturation of cultured *L. seticaudata* through the reduction of water temperature and rearing density. However, both alternatives may decrease profitability in commercial enterprises in that lower water temperatures slow down the growth of cultured juveniles; and individual rearing requires specially designed systems similar to the ones employed to grow clawed lobsters (Nicosia & Lavalli, 1999) and is a much more complex approach than communal shrimp culture. Current achievements in the development of monosex culture (all male) of *Macrobrachium rosenbergii* (Sagi & Aflalo, 2005) may allow a similar approach to be implemented in marine ornamental shrimp culture. All-male culture is considered to be highly advantageous for numerous decapod species, particularly in view of the higher growth rate displayed by these specimens and

because energy is not diverted from growth to reproduction (Curtis & Jones, 1995; Sagi *et al.*, 1997a). Monosex cultures also display a lower ecological risk should an exotic species escape. This aspect is particularly relevant for the culture of marine ornamental shrimps outside their natural distribution area, since it ensures that no accidentally introduced species becomes invasive. A growing body of evidence derived from controlled shrimp breeding trials suggests that the male is the homogametic sex (Sagi & Cohen, 1990; Malecha *et al.*, 1992; Benzie *et al.*, 2001). Through microsurgical intervention it is possible to ablate the androgenic gland of male juvenile shrimps at an early developmental stage. This approach has already been shown to promote the development of functional neo-females in *M. rosenbergii* (Sagi & Cohen, 1990). According to the homogametic male theory (Katakura, 1989), mating neo-females with normal males will result in 100% male progeny. Experimental trials are still required to evaluate the possibility of producing viable neo-females of the most valuable marine ornamental shrimp species and to test the possibility of producing all-male progeny. It is of interest that an ornamental shrimp (*L. seticaudata*) was widely used in preliminary trials addressing the effects of the androgenic hormone on the sexual differentiation of decapod crustaceans (Charniaux-Cotton, 1959a, b, 1960, 1961). Should this approach produce satisfactory results, the trade in neo-females to enterprises producing marine ornamental shrimps may become a new and highly profitable activity.

An alternative way to manipulate sex determination of produced shrimps may be available in the future, namely through the administration at an early stage of the active compounds responsible for the differentiation of masculine sex. As stressed by Sagi & Aflalo (2005), this type of biochemical or molecular manipulation will only be possible after the identification of the exact nature of the androgenic gland hormone (AGH) in decapods. None the less, it must be stressed that biotechnological manipulation, namely at a molecular level, may give rise to some controversy, although it will never be as serious as the one currently surrounding the culture of marine organisms for human consumption (Rasmussen & Morrissey, 2007).

10.4 Traceability and marketability

Traceability may be defined as the ability to ascertain the history, location or use of a specific item by means of an accurate recorded identification. Basically, in the context of marine ornamental species, it should enable anyone at any given point in the chain of custody to know from whom, where, when and how a specific organism (or group of organisms) has been collected/purchased/shipped and to whom, from where, when and how it has been sold/shipped.

The marketing of cultured marine ornamental species is far from being a straightforward issue, as it might at first be thought. In general, the following

marketing aspects will dictate the success of cultured marine ornamentals: the market value of cultured specimens may not be that different from that of wild specimens; how easily cultured specimens can be differentiated from wild ones; and how easily the origin of cultured specimens can be traced by traders and hobbyists. A challenging task for enterprises culturing marine ornamental shrimps is how to differentiate these specimens from shrimps collected in the wild, preventing less scrupulous traders from taking advantage of conscientious hobbyists willing to pay a higher price for cultured specimens. Some producers even feel that captive-cultured specimens, those produced from larvae cultured in captivity, should somehow be distinguished from captive-raised shrimps, those produced from newly settled shrimps collected from the wild. Several tagging techniques have already been successfully used to individually identify shrimps. A simple technique involves the ablation of a conspicuous body part, such as uropods (Leaño & Liao, 2006), that may regenerate but still allow a tagged specimen to be recognized through abnormal development or coloration of that specific body part. Internal monofilament tags and fluorescent elastomer internal tags have also been successfully used to identify juvenile penaeid shrimps (Teboul, 1993; Godin *et al*., 1995). Fluorescent elastomer internal tags have also been used to identify peppermint shrimps *L. wurdemanni* during breeding experiments (Baeza & Bauer, 2004; Baeza, 2007a). However, none of the above tagging options will be a commercially suitable solution to identify cultured marine ornamental shrimps, since tagging would become a labour-intensive and time-consuming operation. This procedure would significantly increase production costs, decrease the marketability of the specimens produced and for more delicate species could even induce mortality. Gallardo-Escárate *et al*. (2007) described how digital images of the color patterns of the rock shrimp *Rhynchocinetes typus* could be used to distinguish between individuals. Despite being a non-intrusive technique, it will only be suitable when identifying a limited number of specimens, being inadequate for commercial-scale production. Rhyne *et al*. (2005) suggested an indirect way to avoid the problems of wild-collected organisms being sold as cultured organisms: focus culture efforts on species that are less common in the trade and/or more expensive to collect. This strategy could be easily followed for two of the most heavily traded marine ornamental shrimp species: *L. amboinensis* and *L. debelius*. Instead of culturing *L. amboinensis*, enterprises should favour the culture of *L. grabhami*, a similar species that displays a different coloration pattern in the tail fan and that is only occasionally available in the trade. *Lysmata splendida* may also be raised in captivity as an alternative to *L. debelius*, since the former species is rarely available for hobbyists and can be distinguished from 'regular' fire shrimps by the presence of several white dots on the dorsal surface of their abdomen. Only wholesalers and/or retailers offering cultured specimens would have large numbers of these 'alternative' species for sale, indirectly reassuring hobbyists that these were not specimens collected from the wild. An additional strategy to indirectly ensure that batches

of shrimp offered for sale are cultured and not collected from the wild is to offer specimens that belong to a single size class. It has already been mentioned that cultured female (or simultaneous hermaphrodite) shrimps are commonly recorded carrying fertilized embryos at significantly smaller sizes than wild specimens. This criterion can also be used to attest to the origin of traded specimens, since only small cultured shrimps will exhibit fertilized embryos in their abdomens and/or visible developing ovaries in the dorsal region of the carapace.

Ultimately, enterprises culturing marine ornamental species should seek certification from international entities, namely the Marine Aquarium Council, which could attest to the production of these organisms. Each batch of traded organisms should be accompanied by a certificate describing relevant information that may allow hobbyists acquiring cultured organisms to track them back to the producing enterprise. Such a policy will only be possible if all members in the chain of custody (producers, wholesalers, retailers and hobbyists) become fully committed to the implementation of the required mechanisms to assure traceability.

Apart from the marine aquarium trade, marine ornamental shrimps cultured in captivity may also be used as biological agents to control nuisance organisms in aquaculture facilities producing marine organisms for human consumption. The most obvious example is probably that of cleaner shrimps. As experimentally demonstrated by Becker & Grutter (2004), cleaner shrimps effectively control the load of fish ectoparasites, namely monogeneans. These ectoparasites readily proliferate and establish large populations in captive fishes held in aquaculture facilities (e.g. Roubal *et al.*, 1992). Employing cleaner shrimps as an alternative control tool to eradicate monogenean pests may be a reliable and less expensive biological alternative to other traditional treatments (e.g. chemicals and freshwater baths). Although cleaner fishes, such as the wrasse *Labroides dimidiatus* Bleeker, 1851, may also be employed (Grutter *et al.*, 2002), the more flexible feeding regime of cleaner shrimps may grant them higher chances of surviving if the loads of ectoparasites significantly decrease and become insufficient to fulfil the dietary needs of cleaners.

Chapter 11

Conservation Issues

Although no destructive techniques are employed in the collection of marine ornamental shrimps, it is not legitimate to say that this is an innocuous activity without any negative impacts on coral reefs or other marine habitats. As with the majority of marine organisms captured for the aquarium trade industry, no study has ever addressed in detail how wild populations of ornamental shrimps are coping with increasing fishing efforts. The possibility of some regions already being overharvested because of a highly localized collection effort is of even greater concern. Indirect evidence of such situations is the smaller size of specimens currently being exported to wholesalers in the European Union (EU). The sizes displayed by these specimens seem to indicate that they are only 2 to 3 months old and, generally, have not yet reached sexual maturity.

The lack of realistic numbers concerning exports of marine ornamental shrimps means that any regulation attempt is destined to be unsuccessful. The remarkable work by Wabnitz *et al.* (2003) clearly underestimates the number of ornamental shrimps being globally traded, probably because only a fraction of enterprises involved in the trade of these organisms (one-fifth according to the authors) actively provided reliable data for the creation of the Global Marine Aquarium Database (GMAD). The first step for sustainable management of these valuable resources will certainly be to determine, as accurately as possible, the number of marine ornamental shrimps being traded annually. Since the majority of these commercial transactions involve air transportation, governmental authorities in importing countries, namely the EU, USA and Japan, already have the necessary legal tools to monitor the origin, the amount and the periodicity of marine ornamental species imports being performed. Current efforts to regulate the trade in marine ornamentals in the EU have already identified two shrimp species whose imports deserve special monitoring. The EU scientific review group on trade in wild fauna recommended that the ornamental shrimps *Lysmata amboinensis* and *L. debelius* should be placed in annex D of the EU Wildlife Trade Regulations (available at http://ec.europa.eu/environment/cites/pdf/srg/31st_summary_srg.pdf). This annex has no equivalent in CITES and includes species that might be

eligible for listing in one of the more restricted annexes (A, B or C, roughly the equivalent to CITES annexes I, II and III) and for which EU import levels are monitored.

According to Grutter (1994) and Côté (2000), the dietary specialization of cleaner organisms has important implications for local fish diversity on coral reefs.

Given that cleaner shrimps have less mobility when compared with cleaner fishes, the location of certain cleaner shrimp species appears to have an even greater potential to affect local fish assemblages (Becker & Grutter, 2004). The ecological implications of harvesting cleaner shrimps, as well as cleaner fishes, must be examined in detail, to ensure that irreparable damage is not inflicted on the reef community. Grutter *et al.* (2002) and Bshary (2003) described how the removal of cleaner fishes promoted a significant decline in fish diversity, which was only significantly noticeable several months later. Becker & Grutter (2004) hypothesized that the negative effects recorded for fish diversity would have been amplified if cleaner shrimps were also removed from the same area. In this way, even when using environmentally friendly fishing techniques, the removal of certain key organisms may indirectly cause significant damage to coral reef communities.

Calado *et al.* (2003c) have drawn attention to the lack of research on popular and pricey marine ornamental decapods, and have pointed out how increasing numbers of these organisms continue to be harvested from the wild in an unmonitored way. However, no study has ever addressed the ecological impact of this practice. While Chockley & St. Mary (2003) addressed the reproductive aspects of the banded coral shrimp *Stenopus hispidus*, Baldwin & Bauer (2003) studied the life cycle of the peppermint shrimp *Lysmata wurdemanni*. The information acquired by such field life-history studies is critical to an understanding of how the populations of these organisms will react to the growing pressure they are facing globally from the trade in marine ornamental species. Curiously, the population biology of the most popular and heavily collected shrimp species, *L. amboinensis* and *L. debelius*, has never been addressed.

Most research efforts addressing marine ornamental shrimps have focused on the development of culture protocols for the most popular species in the industry (e.g. Calado *et al.*, 2003a, 2005a, 2007c). However, it seems evident that, despite all the breakthroughs already achieved in the captive culture of marine ornamental shrimps, the industry will always continue to rely on the collection of wild specimens to fulfil the ever increasing demand for these organisms worldwide. Harriott (2003) suggested that corals may be harvested in a sustainable way as long as suitable management strategies are implemented. Likewise marine ornamental shrimps may be suitable for sustainable collection. The harvesting of abundant species, which also display good growth rates and regular recruitment patterns, may be a suitable option for the long-term use of these valuable resources. Monitoring the most heavily collected species and enforcing the existence of no-take areas are necessary

policies to ensure that sustainable collection is truly accomplished. However, because of the monetary and technical requirements of such measures, coupled with the absence of detailed data on species-specific biological and ecological aspects, it may be difficult to implement such strategies in exporting countries without the commitment of those that import marine ornamentals.

Diversifying the marine ornamental shrimp species available in the trade can be an alternative approach to bring about a decrease in the collection pressure on wild populations of those being regularly supplied to the industry. Additionally, the majority of hobbyists are highly receptive to new species presented in the trade, and the first specimens to be sold always achieve high market values. None the less, recruiting new species for the marine aquarium trade can sometimes have significant negative impacts for the populations of those organisms in the wild. The most well known case study of such a delicate trade-off is that of the Banggai cardinalfish *Pterapogon kauderni* Koumans, 1933. The introduction of this fish in the aquarium trade was so successful that wild populations could not support the fishing efforts required to fulfil the demand, even when employing 'nondestructive' fishing techniques (Kolm & Berglund, 2003). Its reduced reproductive profile, poor larval dispersal ability and restricted distribution make this species too vulnerable to endure the collection pressure imposed by the industry (Allen, 2000; Lunn & Moreau, 2004). Fortunately, commercial-scale protocols for the Banggai cardinalfish are now available (Hopkins *et al*., 2005) and the supply of cultured specimens to the aquarium industry has helped to decrease the pressure on wild populations.

In order to minimize the collecting of peppermint shrimps *Lysmata* from the *wurdemanni* species complex and to provide a suitable alternative to European countries, the culture potential, at a commercial scale, of the autochthonous Monaco shrimp *L. seticaudata* was first evaluated and only afterwards was this species introduced to the hobby. This unique approach set out to present the industry with a regular and large supply of cultured shrimps, avoiding the collection of natural populations and reducing the possibility of creating conservation issues for wild populations (Calado, 2005). Although cultured specimens displayed an attractive coloration and were highly effective in the control of glass anemones *Aiptasia* spp. (Calado & Narciso, 2005), it was only a matter of months until Monaco shrimps collected from the wild entered the aquarium trade in Europe. In some instances wild specimens were even deceptively sold as being cultured in captivity. This is just a single example of the potential conservation issues that southern European countries may be facing in the near future, given the high commercial potential of a large number of species that are now starting to be 'discovered' by the industry (Calado, 2006). The cleaner shrimp *Lysmata grabhami* and the boxer shrimp *Stenopus spinosus* are under threat of being heavily collected in subtropical Atlantic European Islands, as well as Western African tropical countries, if no specific regulations concerning the harvest and trade of marine ornamentals are adopted. Conservation issues that once were thought to be exclusive to countries exporting

marine ornamentals may soon also start to be faced by importing countries in the EU.

If the aquarium industry adopts a minimum size for wild marine ornamental shrimps being traded, more effective conservation of wild populations may be possible. However, establishing minimum sizes is a measure that requires extensive knowledge of the life cycle of target species and will only be effective if it is actively enforced. If such measures are adopted, species being cultured could be sold under the minimum size for wild specimens and like this be easily recognized by hobbyists.

Establishing species quotas and no-take periods for the most heavily collected and traded species is probably one of the most effective and easier to apply management approaches for marine ornamental shrimps. This policy will also increase the chances of cultured organisms entering the trade, even though they may display slightly higher prices, since the industry needs a regular supply of specimens to meet the increasing demand. A good example of how no-take periods favour the marketability of cultured specimens was recorded when cultured Monaco shrimps were presented to the trade. Exhibiting a higher price than Caribbean peppermint shrimps collected from the wild, cultured *L. seticaudata* did not experience immediate popularity among European wholesalers. However, an unusually intense hurricane season in the Caribbean, naturally creating a period hindering the collection of wild specimens, coupled with the need for wholesalers to satisfy their commercial commitments to retailers, gave cultured Monaco shrimps a chance to successfully enter the European marine aquarium trade. In fact, the higher market value displayed by Monaco shrimps was even justified by traders by the fact that these were cultured specimens, and this ultimately promoted the sustainable nature of the marine aquarium hobby.

If the collection of newly settled marine ornamental shrimps becomes a generalized practice, it is possible that governmental agencies will have to adopt management measures similar to the ones already established for the collection of the wild puerulus stage of spiny lobsters. As described by Gardner *et al.* (2006), 'biological neutrality' may be achieved by running a 'quota lease' system, which involves decreasing the fishery quota of marine ornamental shrimps, with the decrease in the number of harvested adult specimens compensating for the removal of newly settled organisms. The lack of current fishing quotas for marine ornamental shrimps makes this option difficult to apply in the short term. The same authors also described an alternative system, the 'reseeding' approach. This strategy involves the release of a percentage of cultured specimens after a grow-out period in captivity, in order to compensate for the collection of organisms that would normally have survived in the wild. If a conservative survival approach is used to determine the percentage of cultured specimens that need to be released after grow-out, an enhancement of wild stocks may actually take place (Gardner *et al.*, 2006). Since released juveniles were originally collected from the wild, the hypothesis of

genetically biasing wild populations is of lesser concern than when performing enhancement operations using specimens hatched in captivity (Gaffney *et al.*, 1996; Doupé & Lymbery, 2000).

Although the accidental release of exotic species from facilities culturing marine ornamental shrimps is unlikely to occur given the fact that most such sites are located inland, this is always a sensitive conservation issue. In fact, marine biological invasions are increasingly considered as a major threat to global biodiversity (Bax *et al.*, 2001; Hewitt & Campbell, 2007). Although the irresponsible and intentional release, associated with episodic escapes, of ornamental species has led to successful freshwater invasions (Courtenay & Stauffer, 1990; Shafland, 1996), the scenario appears to be less concerning in the marine aquarium industry (Calado & Chapman, 2006). Monaco's marine aquarium has been the vector responsible for the invasion of the marine alga *Caulerpa taxifolia* in the Mediterranean (Meinesz & Hesse, 1991; Verlaque & Fritayre, 1994; Jousson *et al.*, 1998), which is far from being over (Cevik *et al.*, 2007). The Pacific lionfish *Pterois volitans* (Linnaeus, 1758) appears to be the only marine ornamental animal species that was able to be successfully established outside its natural distribution area. The first occurrences in the Atlantic of *P. volitans* were documented by Whitfield *et al.* (2002), and irrefutable evidence of successful reproductive behavior of these invasive specimens was provided by Ruiz-Carus *et al.* (2006). Semmens *et al.* (2004) drew attention to the existence of 'hotspots of non-native marine fishes' (e.g. Florida) as a direct consequence of the aquarium trade, with less conscientious traders using near-shore facilities being responsible for the majority of recorded invasions. The threats to coastal ecosystems by invading marine ornamentals are largely unpredictable and, once detected, rapid proactive measures for the eradication or control of their expansion must be implemented by governmental managers and scientists.

The creation of an 'eco-fee' to tax imported wild-caught marine ornamental species could help to finance national and/or international marine ornamental research and management policies. As little as 1% of the retail value of each traded marine ornamental organism collected from the wild would allow a considerable amount of funding to be allocated to the management of marine ornamental fisheries, as well as to finance research efforts to develop culture protocols for these highly valuable resources. This 'eco-fee' would reassure conscientious hobbyists that they are indeed contributing to the conservation and sustainable management of marine resources.

Sharing the acquired know-how and providing technical training for the culture of marine ornamentals to local populations involved in the collection and trade of marine ornamentals in exporting countries must always be a priority to importing countries.

The socio-economic dependence of these populations on the trade in marine ornamental species has made them oppose the culture of these organisms, considering this activity as a potential threat to their daily subsistence

(Watson, 2000; Tlusty, 2002). Their major concern is the fact that most culture operations will not be developed in their local regions, with production probably even being shifted to importing countries and putting at risk one of the few ways these populations have to overcome their extreme poverty. If aquacultural programmes are designed to replace, or in a more realistic vision decrease, the collection of wild specimens, local populations must get actively involved or these initiatives will be destined to have little or no impact on ecosystem conservation.

With adequate funding and technical assistance during the initial period, small-scale aquaculture of marine ornamentals can be a feasible and profitable activity (Pomeroy & Balboa, 2004). The selection of highly profitable species, such as cleaner shrimps *Lysmata* spp. and boxer shrimps *Stenopus* spp., are vital for the success of such endeavours, with importing countries having the moral obligation to fully support such initiatives.

As remarked by Tlusty (2004), marine ornamentals aquaculture is in a privileged position as it is able to learn from the wide range of successes and constraints faced by freshwater ornamental and food-fish aquaculture. This expanding new sector of marine aquaculture can and must develop a suitable code of conduct, since this is a vital tool to overcome future difficulties that will certainly take place if it continues to grow so rapidly without a well-defined strategy.

Certification programmes similar to the ones currently being developed by the Marine Aquarium Council will certainly play a vital role in the conservation of marine ornamental species. The wide scope of action of such certification programmes, which require the bringing together of collectors, traders, researchers and hobbyists, reinforces the complexity of such initiatives.

The importance of traceability in the entire chain of custody allows organisms that were collected and/or produced, stocked and shipped according to sustainable practices to be marketed with a quality label, significantly increasing their economic value.

If on the one hand it is necessary to assure the sustainability of collection and/or production practices, it is also vital for the marine aquarium industry to ensure that hobbyists understand and support such certification programmes. Only by turning anonymous hobbyists into conscientious consumers, willing to prefer eco-friendly marine ornamentals, will any certification and/or conservation programme be truly successful. Ultimately, the future of the marine aquarium industry is entirely dependent on the concerns of hobbyists regarding the sustainable and rational use of these highly priced resources.

Chapter 12

Conclusions

The culture of ornamental species will certainly be one of the sectors of marine aquaculture that will experience accelerated expansion in the coming years. It is remarkable how, in less than a decade, marine ornamental shrimp culture passed from a possibility to a profitable reality. Obviously, there is still room for improvement. In order to overcome existing bottlenecks, truly multidisciplinary teams need to address the biology, aquaculture and conservation of marine ornamental shrimps.

Current research efforts are too biased towards the development of suitable culture protocols. However, finding relevant information on the biological and ecological habits of the most heavily traded species remains to be addressed. Field marine biologists studying population dynamics can play a crucial role by providing information on the population structure of marine ornamental shrimps in the wild. Reliable data on the onset of sexual maturation, gene flow between neighbouring populations, recruitment studies and growth rates are essential to determine reliable management measures to regulate the collection and trade of marine ornamental shrimps. Such studies require considerable time and financial investment, and it would be unrealistic to address all traded shrimp species. Since the most heavily collected species are also the ones reaching higher market values, field studies should focus on those organisms. Recorded information would be valuable not only for the management of wild populations, but also to optimize captive culture methodologies. Maturation protocols would greatly benefit from a knowledge of the average number of embryos produced by females in the wild, as well as of eventual egg losses during the incubation period. These data would allow researchers to evaluate the performance of broodstock diets used in captivity, with the aim of matching or even improving embryo production in captivity. Additional information on the biochemical composition of developing embryos in the wild would make it possible to adjust broodstock diets and maximize the quality of egg clutches produced in captivity.

Information on size at the onset of sexual maturation allows policy makers to establish minimum size for wild shrimp collection. By knowing recruitment patterns and understanding gene flow dynamics between neighbouring

populations, it will also be possible to establish reasonable fishing quotas and decide which collection sites will be more vulnerable to overfishing. Additionally, these studies are also vital to ensure that collecting newly settled shrimps from the wild, for subsequent grow-out in captivity, can truly be a sustainable alternative to current techniques requiring larval culture.

Knowledge of growth rates of wild shrimps can help researchers to improve grow-out diets and thereby to maximize production and increase profitability.

Marine planktologists may also provide valuable contributions to researchers addressing the larval culture of marine ornamental shrimps. Detailed morphological descriptions of the larval stages of marine ornamental shrimps will allow researchers to accurately monitor the development period of different zoeal stages. It would then be possible to diagnose the existence of unsuitable rearing conditions by recording abnormal larval stage durations (mark-time moulting). It is still necessary to study in detail the morphological changes occurring in the feeding structures of marine ornamental shrimp larvae during their zoeal development. Scanning electron micrographs of the mouthparts of different zoeal stages of ornamental shrimps would allow researchers to identify shifts in their preference for different-sized prey and food textures and their ability to manipulate food items. Through the use of high-speed video analysis it will be possible to understand which larval appendages are involved in prey detection, capture and manipulation, allowing a better evaluation of alternative live and inert dietary items.

The microbiological analysis of marine ornamental shrimp larvae collected from the plankton provides researchers with information on which microorganisms may act as probiotic agents during larval culture trials, namely for those species exhibiting long larval cycles. Through the DNA analysis of larval stomach contents it may be possible to identify dietary prey items of late zoeal stages. The use of fatty acid trophic markers may also provide valuable insights into the preferred prey of ornamental shrimp larvae in the plankton. Larval natural diets, or at least their biochemical profiles, may be mimicked in captivity and reduce the larval period until metamorphosis.

With suitable culture systems already developed, although minor species-specific adjustments may still be required, the larval culture of marine ornamental shrimps will certainly benefit from the intensive research efforts that continue to be performed to maximize the larval production of other decapod crustaceans used for human consumption. The permanent search in aquaculture for alternative live dietary prey smaller than rotifers and larger than *Artemia* metanauplii, as well as for attractive formulated diets for larval decapods capable of partially or even fully replacing live diets, will certainly improve the culture of marine ornamental shrimps.

The development of grow-out inert diets will certainly shorten the period required to achieve commercial size, will significantly improve survival through the minimization (or even elimination) of cannibalism, and will

reduce production costs. Research studies on sex determination, through the manipulation of the androgenic gland, will make monosex cultures a reality in the coming years. For the culture of marine ornamental shrimps there will be the possibility of culturing all-male batches displaying faster growth rates to marketable size. It will also mean that, if cultured specimens of an exotic species escape to the wild, there will be a lower risk of bio-invasion.

The large-scale production of species only occasionally available in the aquarium trade, either because they are difficult to collect or because they occur in less accessible geographical areas, will be the best way to ensure that wild specimens will not be sold as captive cultured. Traceability of cultured organisms, from the hobbyist to the producer, will play a major role in their marketability, particularly if they are being produced in a region where wild specimens can be easily harvested.

Ultimately, researchers addressing the biology, aquaculture and/or conservation of marine ornamental shrimps must tune their efforts to the needs expressed by traders, collectors, policy makers and hobbyists. Consensual approaches involving all the partners in the chain of custody will certainly result in higher success than will individual attempts that ignore the reality of the marine aquarium industry. Importing countries should be the main promoters of research programmes promoting the sustainable use of marine ornamental organisms. These initiatives should be implemented in exporting countries, involving local populations and ensuring that these have a relevant role in the development of such programmes. Any management decision affecting the revenues generated by the collection and trade of marine ornamentals must be carefully evaluated since the social impact of any precipitate measures can be devastating.

It is the responsibility of all of those who love marine aquariums to ensure that marine ornamental shrimps, as well as all other traded organisms, continue to be available to hobbyists for many years to come and that they can also be observed in their natural environment by all of those willing to admire the beauty of these organisms in the wild.

Glossary

Alkalinity Measures the resistance of a solution to pH variation when an acid solution is added.

Androgenic gland A holocrine gland (the entire cell disintegrates to secrete its substance) found in most malacostracan crustaceans and responsible for the production of hormones controlling the development of testes and male sexual characteristics.

Appendix interna A finger-like organ located in the endopod of shrimp pleopods; its distal end is covered with tiny hooks called cincinulli.

Appendix masculinae A finger-like organ located in the endopod of the second pair of pleopods of male shrimps, with its distal end, and sometimes the ventral surface, covered with broad spines. It degenerates in protandric species when the shrimp changes from male phase to female or simultaneous hermaphrodite phase.

Artemia Also known as brine shrimp, it is a relatively primitive form of crustacean of the class Brachiopoda. It has the ability to produce cysts (embryos whose development is temporarily 'paused' and which are encased in a complex shell), which are commonly traded in vacuum-packed cans. These cysts can be easily incubated to produce newly hatched *Artemia* nauplii, a very popular feed for marine larvae. Live, frozen, freeze-dried adult or newly hatched *Artemia* is commonly used as food for marine organisms.

Autotomy Also designated as self-amputation, this is the act whereby an animal severs one of its own appendages, usually as a self-defence mechanism designed to avoid a predator's grasp.

Buffer A solution that is resistant to pH changes despite the addition of small amounts of acid or basic solutions.

Caridea An infraorder of decapod crustaceans which includes all shrimps incubating their eggs under the abdomen. These may be differentiated from stenopodidean shrimps by the absence of chelae in the third pair of pereiopods and from penaeid shrimps by incubating their eggs in the abdomen and by the pleura of their second abdominal segment overlapping those of the first and third segments.

Decapodid A term proposed by Kaestner (1980) to denote the final larval phase preceding metamorphosis to the first juvenile stage. It is used as an alternative to megalopa.

Dendrobranchiata A sub-order of decapod crustaceans which includes penaeoid and sergestoid shrimps. These shrimps do not incubate their fertilized eggs in the pleopods until hatching. Their planktonic eggs hatch as nauplii and not as zoeae.

Denitrification The process of conversion of nitrate to nitrogen gas through the action of anaerobic bacteria.

Ecdysis A termed used to designate the process of shedding the old exoskeleton by a decapod crustacean (or any other arthropod), a prerequisite to development and growth.

Ex situ The opposite of *in situ*, it means to examine a given process outside the place where it occurs (e.g. in the laboratory).

Exuvia The name given to the old exoskeleton that is shed by decapod crustacean (or any other arthropod) during ecdysis. Occasionally, certain morphological aspects, such as secondary sexual features, may be more easily observed in the exuvia because of its translucent appearance.

Glaucothoe The last larval stage of hermit crabs, corresponding to the megalopa of other decapod crustaceans.

GMAD Global Marine Aquarium Database, a freely available source of information on the global aquarium industry developed by the United Nations Environment Program — World Conservation Monitoring Centre (UNEP–WCMC) and the Marine Aquarium Council (MAC) in collaboration with members of various marine ornamental species trade associations (http://www.unep-wcmc.org/marine/GMAD/index.html).

Gonochoric A shrimp species displaying separate sexes.

Gonopore A term used to designate the genital pore, which is located in the base of the fifth pair of walking legs in males and at the base of the third pair of walking legs in females.

In situ The opposite of *ex situ*, it means to examine a given process exactly in the place where it occurs (e.g. in the ocean).

Intercalated stages An event that increases the length of the pelagic larval phase of the species. It can be defined as the insertion of extra stages between those normally occurring in a species developmental series.

Larval settlement Transition to benthic life of pelagic free-swimming larvae.

Lecithotrophy In opposition to planktotrophy, larvae displaying this mode of development do not require food to survive, relying on the degradation of internal reserves to fulfil energetic requirements. It is commonly considered an adaptation to low or unpredictable food levels in the environment in which larval development takes place.

Live rock Is the term used to designate the rock made from the calcium carbonate skeletons of long dead corals, or other calcareous organisms, forming the majority of coral reefs in the ocean. These rocks are commonly

encrusted with coralline algae and inhabited by a multitude of marine organisms. The various forms of micro- and macroscopic life living on and in the rock give it the name 'live rock'.

MAC Marine Aquarium Council, an international, not-for-profit organization that brings marine aquarium animal collectors, exporters, importers and retailers together with aquarium keepers, public aquariums, conservation organizations and government agencies. Its mission is to conserve coral reefs and other marine ecosystems by creating standards and certification for those engaged in the collection and care of ornamental marine life from reef to aquarium (www.aquariumcouncil.org).

Mark-time moulting An event that increases the pelagic larval phase of the species. It occurs when a zoeal stage enters a sequence of moults in which very little change in morphology takes place, even if it exhibits some small increases in larval size.

Megalopa A term commonly used to designate the final larval phase preceding metamorphosis in brachyuran crabs, which has also been proposed for all decapod crustaceans by Williamson (1969). It is characterized by the presence of functional swimming pleopods, while all cephalic and anterior thoracic appendages assume new functions. It is used as an alternative to decapodid.

Metamorphosis In the strict sense it describes a particular life history transition from a larval to a juvenile (or adult) stage, accompanied by dramatic modifications in its morphology, physiology and ecology [see Bishop *et al.* (2006) for an overview].

Metanauplius This larval stage is very similar to the nauplius, also displaying a median eye and cephalic natatory appendages. However, it has more than three somites and may also present thoracic appendages.

Microalgae They comprise a vast group of photosynthetic, heterotrophic organisms that may be directly used as food for marine larvae or to improve the nutritional profile of other larval prey (e.g. *Artemia* and rotifers).

Mysis A term proposed by Gurney (1942) to describe the zoeal stages of penaeid and sergestoid shrimps beyond the fourth larval stage. It has become widely popular among aquaculturists raising penaeids.

Nauplius Is considered the most ancestral type of larvae in the Crustacea. In the Decapoda it only occurs as a free-swimming stage in the Dendrobranchiata, while all other 'higher' decapods pass through this stage during embryonic development. It displays a median eye, has no thoracic somites and its locomotion is achieved through the use of cephalic appendages (namely the antennules, antennae and mandibles).

Nitrification A two-step process in which ammonia is converted to nitrite and later to nitrate by nitrifying bacteria.

pH A measure of the acidity or alkalinity of a solution. Solutions with a pH less than 7.0 are considered acidic, while those with a pH greater

than 7.0 are considered basic (or alkaline). When a pH level is 7.0, it is defined as 'neutral'.

Phototactism Concerns the behavior of an organism in the presence of light. It can be positive if it moves towards the light source or negative if it moves away from it.

Phyllosoma The larval stage of spiny lobsters, corresponding to the zoea of other decapod crustaceans.

Planktotrophy The opposite of lecithotrophy: larvae displaying this mode of development require planktonic food to survive and fulfil their energetic requirements.

Pleocyemata A sub-order of decapod crustaceans which includes all non-penaeid decapods, whether swimming (natant) or crawling (reptant) (stenopodid and caridean shrimps, crabs, hermit crabs, lobsters, crayfish, and others). All these taxa are united by the fact that females incubate their fertilized eggs in the pleopods until hatching.

Point of no return This critical point within larval development was first designated by Blaxter & Hempel (1963) and represents a threshold at which starved and subsequently fed larvae may remain alive for a given period, cannot recover from nutritional stress, lose their ability to develop, and die. Its existence was confirmed in decapods by Anger & Dawirs (1981).

Point of reserve saturation A critical point within larval development beyond which food uptake is no longer essential for subsequent development and moulting to the following larval stage, as described by Anger & Dawirs (1981).

Post-larvae This term has been considered by Williamson (1969) as 'illogical as a noun' since it implies a non-larval nature but it is often applied to both larval and early juvenile stages. None the less, this term is still widely used in aquaculture although it is often uncertain as to whether it concerns the last larval stage or earlier juvenile stages.

Primary lecithotrophy Larvae displaying this mode of development use energy derived from endogenous egg yolk reserves to develop through some or all post-embryonic larval stages. This mode of development commonly reflects the degree of female investment in reproduction.

Protandric A shrimp that first matures as a male and with increasing size may lose the external male features; it stops producing sperm and becomes a functional female.

Protandric simultaneous hermaphrodite A shrimp that first matures as male and with increasing size may lose the external male characteristics and change into a euhermaphrodite. A euhermaphrodite-phase shrimp is able to mate as a female (immediately after the moult), as well as a male (during intermoult), but is unable to perform self-fertilization.

Puerulus The last larval stage of spiny lobsters, corresponding to the megalopa of other decapod crustaceans.

Reef safe Term used by marine aquarium hobbyists to label a species that will not damage other organisms being displayed in the same reef aquarium.

Rotifers These are microscopic aquatic animals of the phylum Rotifera. The rotifer *Brachionus plicatilis* has been widely used as food for larval marine organisms due to its tolerance to the marine environment. It is commonly used to feed larval stages which have no or limited ability to capture and ingest *Artemia* nauplii.

Secondary lecithotrophy A development mode displayed by a late non-feeding larval stage, which will moult to a juvenile that resumes feeding activity. During this period the energetic requirements are fulfilled through the catabolism of plankton-derived reserves accumulated by earlier larval stages.

Stenopodidea An infraorder of decapod crustaceans which includes all stenopodidean shrimps. These may be differentiated from caridean shrimps by the existence of well-developed chelae in their third pair of pereiopods and from penaeid shrimps through the incubation of their eggs under the abdomen.

Terminally additive stages An event that increases the pelagic larval phase of the species. It can be defined as a particular case of mark-time moulting which only takes place at the end of a developmental sequence.

UNEP–WCMC United Nations Environment Program — World Conservation Monitoring Centre, a collaboration between the United Nations Environment Programme, the world's foremost intergovernmental organization, and WCMC 2000, a UK-based charity (www.unep-wcmc.org).

Zoea This larval stage is more advanced than the nauplius (with caridean and stenopodidean shrimps hatching as a zoea) and can be easily diagnosed by the presence of functional thoracopods and paired compound eyes. The antennules and the antennae assume sensorial functions, while the exopods of thoracic appendages fulfil the natatory functions. The pleopods are generally present in later stages, either as rudimentary buds or as biramous leaf-like appendages, but never display a natatory function.

References

Abdel-Rahman, S.H., Kanazawa, A. & Teshima, S.I. (1979) Effect of dietary carbohydrate on the growth and the levels of the hepatopancreatic glycogen and serum glucose of prawn. *Bulletin of the Japanese Society of Scientific Fisheries*, **45**, 1491–1494.

Abdu, U., Davis, C., Khalaila, I. & Sagi, A. (2002) The vitellogenin cDNA of *Cherax quadricarinatus* encodes a lipoprotein with calcium binding ability, and its expression is induced following the removal of the androgenic gland in a sexually plastic system. *General and Comparative Endocrinology*, **127**, 263–272.

Abele, L.G. (1970) Semi-terrestrial shrimp (*Merguia rhizophorae*). *Nature*, **226**, 661–662.

Abelló, P., Visauta, E., Bucci, A. & Demestre, M. (2003) Noves dades sobre l'expansió del cranc *Percnon gibbesi* (Brachyura: Grapsidae: Plagusiinae) a la Mediterrània occidental. *Bolleti de la Societat d'Historia Natural de les Balears*, **46**, 73–77.

Achituv, Y. & Pedrotti, M.L. (1999) Costs and gains of porcelain crab suspension feeding in different flow conditions. *Marine Ecology Progress Series*, **184**, 161–169.

Ackefors, H., Castell, J.D., Boston, L.D., Raty, P. & Svensson, M. (1992) Standard experimental diets for crustacean nutrition research: II. Growth and survival of juvenile crayfish *Astacus astacus* (Linne) fed diets containing various amounts of protein, carbohydrate and lipid. *Aquaculture*, **104**, 341–356.

Adams, C.F. & Paul, A.J. (1999) Phototaxis and geotaxis of light-adapted zoeae of the golden king crab *Lithodes aequispinus* (Anomura: Lithodidae) in the laboratory. *Journal of Crustacean Biology*, **19**, 106–110.

Ahvenharju, T. & Ruohonen, K. (2007) Agonistic behaviour of signal crayfish (*Pacifastacus leniusculus* Dana) in different social environments: effect of size heterogeneity on growth and food intake. *Aquaculture*, **271**, 307–318.

Ahyong, S.T. & O'Meally, D. (2004) Phylogeny of the Decapoda Reptantia: resolution using three molecular loci and morphology. *The Raffles Bulletin of Zoology*, **52**, 673–693.

Alabi, A.O., Che Cob, Z., Jones, D.A. & Latchford, J.W. (1999) Influence of algal exudates and bacteria on growth and survival of white shrimp larvae fed entirely on microencapsulated diets. *Aquaculture International*, **7**, 137–158.

Alava, V.R., Kanazawa, A., Teshima, S. & Koshio, S. (1993a) Effect of dietary phospholipids and n-3 highly unsaturated fatty acids on ovarian development of Kuruma prawn. *Nippon Suisan Gakkaishi*, **59**, 345–351.

Alava, V.R., Kanazawa, A., Teshima, S. & Koshio, S. (1993b) Effect of dietary vitamins A, E and C on the ovarian development of *Penaeus japonicus*. *Nippon Suisan Gakkaishi*, **59**, 1235–1241.

Allen, G.R. (2000) Threatened fishes of the world: *Pterapogon kaudneri* Koumans 1933 (Apogonidae). *Environmental Biology of Fishes*, **57**, 142.

Alvandi, S.V., Vijayan, K.K., Santiago, T.C., Poornima, M., Jithendran, K.P., Ali, S.A. & Rajan, J.J.S. (2004) Evaluation of *Pseudomonas* sp. PM 11 and *Vibrio fluvialis* PM 17 on immune indices of tiger shrimp, *Penaeus monodon*. *Fish and Shellfish Immunology*, **17**, 115–120.

Anderson, G. (1990) Postinfection mortality of *Palaemonetes* spp. (Decapoda: Palaemonidae) following experimental exposure to the bopyrid isopod *Probopyrus pandalicola* (Packard) (Isopoda: Epicaridea). *Journal of Crustacean Biology*, **10**, 284–292.

Anger, K. (1989) Growth and exuvial loss during larval and early juvenile development of the hermit crab *Pagurus bernhardus* reared in the laboratory. *Marine Biology*, **103**, 503–511.

Anger, K. (1995) Starvation resistance in larvae of a semiterrestrial crab, *Sesarma curacaoense* (Decapoda: Grapsidae). *Journal of Experimental Marine Biology and Ecology*, **187**, 161–174.

Anger, K. (2001) *The Biology of Decapod Crustacean Larvae. Crustacean Issues 14.* A.A. Balkema, Lisse, The Netherlands.

Anger, K. (2006) Contributions of larval biology to crustacean research: a review. *Invertebrate Reproduction and Development*, **49**, 175–205.

Anger, K. & Dawirs, R. (1981) Influence of starvation on the larval development of *Hyas araneus* (Decapoda, Majidae). *Helgoländer wissenschaften Meeresuntersuchen*, **34**, 287–311.

Anger, K. & Puschel, C. (1986) Growth and exuviation of Norway lobster (*Nephrops norvegicus*) larvae reared in the laboratory. *Ophelia*, **25**, 157–167.

Anker, A., Jeng, M.S. & Chan, T.Y. (2001) Two unusual species of Alpheidae (Decapoda: Caridea), associated with upogebiid mudshrimps in the mudflats of Taiwan and Vietnam. *Journal of Crustacean Biology*, **21**, 1049–1061.

Anker, A., Murina, G.V., Lira, C., Vera Caripe, J.A., Palmer, A.R. & Jeng, M.S. (2005) Macrofauna associated with echiuran burrows: a review with new observations of the innkeeper worm, *Ochetostoma erythrogrammon* Leuckart and Rüppel, in Venezuela. *Zoological Studies*, **44**, 157–190.

Anker, A., Ahyong, S.T., Noël, P.Y. & Palmer, A.R. (2006) Morphological phylogeny of alpheid shrimps: parallel preadaptation and the origin of a key morphological innovation, the snapping claw. *Evolution*, **60**, 2507–2528.

Argue, B.J., Arce, S.M., Lotz, J.M. & Moss, S.M. (2002) Selective breeding of Pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura Syndrome Virus. *Aquaculture*, **204**, 447–460.

Arnold, S.J., Sellars, M.J., Crocos, P.J. & Coman, G.J. (2006) Intensive production of juvenile tiger shrimp *Penaeus monodon*: an evaluation of stocking density and artificial substrates. *Aquaculture*, **261**, 890–896.

Austin, B. & Allen, D.A. (1982) Microbiology of laboratory-hatched brine shrimp (Artemia). *Aquaculture*, **26**, 369–383.

Aziz, K. & Greenwood, J. (1982) Response of juvenile *Metapenaeus bennetae* Racek & Dall, 1965 (Decapoda, Penaeidae) to sediments of differing particle size. *Crustaceana*, **43**, 121–126.

Baeza, J.A. (2006) Testing three models on the adaptive significance of protandric simultaneous hermaphroditism in a marine shrimp. *Evolution*, **60**, 1840–1850.

Baeza, J.A. (2007a) Male mating opportunities affect sex allocation in a protandric–simultaneous hermaphroditic shrimp. *Behavioral Ecology and Sociobiology*, **61**, 365–370.

Baeza, J.A. (2007b) Sex allocation in a simultaneously hermaphroditic marine shrimp. *Evolution*, **61**, 2360–2373.

Baeza, J.A. (2007c) No effect of group size on sex allocation in a protandric–simultaneous hermaphroditic shrimp. *Journal of the Marine Biological Association of the United Kingdom*, **87**, 1169–1174.

Baeza, J.A. & Bauer, R.T. (2004) Experimental test of socially mediated sex change in a protandric simultaneous hermaphrodite, the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae). *Behavioural Ecology and Sociobiology*, **55**, 544–550.

Baeza, J.A. & Stotz, W.B. (2001) Host-use pattern and host-selection during ontogeny of the commensal crab *Allopetrolisthes spinifrons* (H. Milne Edwards, 1837) (Decapoda: Anomura: Porcellanidae). *Journal of Natural History*, **35**, 341–355.

Baeza, J.A. & Thiel, M. (2003) Predicting territorial behavior in symbiotic crabs using host characteristics: a comparative study and proposal of a model. *Marine Biology*, **142**, 93–100.

Baeza, J.A., Stotz, W. & Thiel, M. (2002) The agonistic behaviour and development of territoriality during ontogeny of the sea anemone dwelling crab *Allopetrolisthes spinifrons*. *Marine and Freshwater Behaviour and Physiology*, **35**, 189–202.

Baisre, J.A. (1969) A note on the phyllosoma *Justitia longimanus* (H. Milne Edwards) (Decapoda, Palinuridea). *Crustaceana*, **16**, 182–184.

Baldwin, A.P. & Bauer, R.T. (2003) Growth, survivorship, life-span, and sex change in the hermaphroditic shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *Marine Biology*, **143**, 157–166.

Ballester, E.L.C., Wasielesky W., Jr., Cavalli, R.O. & Abreu, P.C. (2007) Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: biofilm composition and shrimp performance. *Aquaculture*, **269**, 355–362.

Banner, A.H. & Banner, D.M. (1983) An annotated checklist of the alpheid shrimp from the western Indian Ocean. *Travaux et Documents de l'ORSTOM*, **158**, 1–164.

Baquero, J. (1995) The stressful journey of marine ornamental fish. *Ocean Voice International Bulletin, SeaWind*, **9**, 19–21.

Barki, A., Karplus, I., Manor, R., Parnes, S., Aflalo, E.D. & Sagi, A. (2006) Growth of redclaw crayfish (*Cherax quadricarinatus*) in a three-dimensional compartments system: does a neighbor matter? *Aquaculture*, **252**, 348–355.

Barnich, R. (1996) *The Larvae of the Crustacea Decapoda (excl. Brachyura) in the Plankton of the French Mediterranean Coast (identification keys and systematic review)*. Cuvillier Verlag, Göttingen, Germany.

Barros, H.P. & Valenti, W.C. (2003) Food intake in *Macrobrachium rosenbergii* during larval development. *Aquaculture*, **216**, 165–176.

Bartilotti, C., Calado, R. & dos Santos, A. (in press) Complete larval development of the hermit crabs *Clibanarius aequabilis* and *Clibanarius erythropus* (Decapoda: Anomura: Diogenidae), under laboratory conditions, with a revision of the larval features of genus *Clibanarius*. *Helgoland Marine Research*.

Bauer, R.T. (1976) Mating behaviour and spermatophore transfer in the shrimp *Heptacarpus pictus* (Stimpson) (Decapoda: Caridea: Hippolytidae). *Journal of Natural History*, **10**, 415–440.

Bauer, R.T. (1979) Antifouling adaptations of marine shrimp (Decapoda: Caridea): gill cleaning mechanisms and grooming of brooded embryos. *Marine Biology*, **49**, 69–82.

Bauer, R.T. (1981a) Grooming behavior and morphology in the decapod Crustacea. *Journal of Crustacean Biology*, **1**, 153–173.

Bauer, R.T. (1981b) Color patterns of the shrimps *Heptacarpus pictus* and *H. paludicola* (Caridea: Hippolytidae). *Marine Biology*, **64**, 141–152.

Bauer, R.T. (1986) Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. *Journal of Crustacean Biology*, **6**, 313–325.

Bauer, R.T. (1989) Continuous reproduction and episodic recruitment in nine shrimp species inhabiting a tropical seagrass meadow. *Journal of Experimental Marine Biology and Ecology*, **127**, 175–187.

Bauer, R.T. (1991) Analysis of embryo production in a caridean shrimp guild from a tropical seagrass meadow. In: *Crustacean Issues*, Vol. 7 (Crustacean Egg Production) (eds A. Wenner & A. Kuris) pp. 181–191. A.A. Balkema, Rotterdam.

Bauer, R.T. (2000) Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. *Journal of Crustacean Biology*, 20, special issue 2, 116–128.

Bauer, R.T. (2005) Cost of maleness on brood production in the shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae), a protandric simultaneous hermaphrodite. *Journal of the Marine Biological Association of the United Kingdom*, **85**, 101–106.

Bauer, R.T. (2006) Same sexual system but variable sociobiology: evolution of protandric simultaneous hermaphroditism in *Lysmata* shrimps. *Integrative and Comparative Biology*, **46**, 430–438.

Bauer, R.T. & Holt, G.J. (1998) Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae): an undescribed sexual system in the decapod Crustacea. *Marine Biology*, **132**, 223–235.

Bauer, R.T. & Min, L.J. (1993) Spermatophores and plug substances of the marine shrimp *Trachypenaeus similes* (Crustacea: Decapoda: Penaidae): formation in the male reproductive tract and disposition in the inseminated female. *Biological Bulletin*, **185**, 174–185.

Bauer, R.T. & Newman, W.A. (2004) Protandric simultaneous hermaphroditism in the marine shrimp *Lysmata californica* (Caridea: Hippolytidae). *Journal of Crustacean Biology*, **24**, 131–139.

Bauer, R.T. & VanHoy, R. (1996) Variation in sexual systems (protandry, gonochorism) and reproductive biology among three species of the shrimp genus *Thor* (Decapoda: Caridea). *Bulletin of Marine Science*, **59**, 53–73.

Bautista, M.N. (1986) The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. *Aquaculture*, **53**, 229–242.

Bax, N., Carlton, J.T., Mathews-Amos, A., Haedrich, R.L., Hogwarth, F.G., Purcell, J.E., Rieser, A. & Gray, A. (2001) The control of biological invasions in the world's oceans. *Conservation Biology*, **15**, 1234–1246.

Becker, J.H.A. & Grutter, A.S. (2004) Cleaner shrimp do clean. *Coral Reefs*, **23**, 515–520.

Becker, J.H.A. & Grutter, A.S. (2005) Client fish ectoparasite loads and cleaner shrimp *Urocaridella* sp. c hunger levels affect cleaning behaviour. *Animal Behaviour*, **70**, 991–996.

Becker, J.H.A., Curtis, L.M. & Grutter A.S. (2005) Cleaner shrimp use a rocking dance to advertise cleaning service to clients. *Current Biology*, **15**, 760–764.

Bedini, R., Canali, M.G. & Bedini, A. (2002) Functions of the eyespot marking on the swimming leg of *Liocarcinus depurator* (Linnaeus, 1758) (Decapoda, Brachyura, Portunidae). *Crustaceana*, **75**, 979–992.

Bell, J.G., McEvoy, L.A., Estevez, A. & Sargent, J.R. (2003) Optimizing lipid nutrition in first-feeding flatfish larvae. *Aquaculture*, **227**, 211–220.

Ben-Amotz, A., Fishler, R. & Schneller, A. (1987) Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. *Marine Biology*, **95**, 31–36.

Benayoun, G. & Fowler, S. (1980) Long-term observations on the moulting frequency of the shrimp *Lysmata seticaudata*. *Marine Biology*, **59**, 219–223.

Bengtson, D.A., Léger, P. & Sorgeloos, P. (1991) Use of *Artemia* as a food source for aquaculture. In: *Artemia Biology* (eds R.A. Browne, P. Sorgeloos & C.N.A. Trotman) pp. 255–285. CRC Press Inc, Boca Raton, Florida, USA.

Benijts, F., Vanvoorden, E. & Sorgeloos, P. (1976) Changes in the biochemical composition of the early larval stages of the brine shrimp, *Artemia salina* L. In: *Proceedings of the 10th European Symposium on Marine Biology*, Vol. I (Research in Mariculture at Laboratory and Pilot Scale) (eds G. Persoone & E. Jaspers) pp. 1–9. Universa Press, Wetteren, Belgium.

Benvenuto, C. & Gherardi, F. (2001) Population structure and shell use in the hermit crab, *Clibanarius erythropus*: a comparison between Mediterranean and Atlantic shores. *Journal of the Marine Biological Association of the United Kingdom*, **81**, 77–84.

Benzie, J.A.H. (1998) Penaeid genetics and biotechnology. *Aquaculture*, **164**, 23–47.

Benzie, J.A.H., Kenway, M. & Ballment, E. (2001) Growth of *Penaeus monodon* X *Penaeus esculentus* tiger prawn hybrids relative to the parental species. *Aquaculture*, **193**, 227–237.

Berg, A.B. & Sandifer, P. (1984) Mating behavior of the grass shrimp *Palaemonetes pugio* Holthuis (Decapoda, Caridea). *Journal of Crustacean Biology*, **4**, 417–424.

Bergström, B.I. (2000) The biology of Pandalus. In: *Advances in Marine Biology* (eds A.J. Southward, P.A. Tyler, C.M. Young & L. Fuiman) pp. 55–244. Academic Press, London, UK.

Berkes, F. (1975) Some aspects of feeding mechanisms of euphausiid crustaceans. *Crustaceana*, **29**, 266–270.

Berreur-Bonnenfant, J. & Lawrence, F. (1984) Comparative effect of farnesylacetone on macromolecular synthesis in gonads of crustaceans. *General and Comparative Endocrinology*, **54**, 462–468.

Berreur-Bonnenfant, J., Meusy, J.J., Ferezou, J.P., Devys, M., Quesneau-Thierry, A. & Barbier, M. (1973) Secretion of the androgenic gland of malacostracan Crustacea: purification of substance with androgenic activity. *Comptes Rendus de l'Academie des Sciences Paris*, **277**, 971–974.

Bertness, M.D. (1981) The influence of shell type on hermit crab growth rate and clutch size (Decapoda, Anomura). *Crustaceana*, **40**, 197–205.

Biedenbach, J.M., Smith, L.L. & Lawrence, A.L. (1990) Use of a new spray-dried algal product in penaeid larviculture. *Aquaculture*, **86**, 249–257.

Bigford, T.E. (1979) Ontogeny of light and gravity responses in rock crab larvae (*Cancer irroratus*). *Marine Biology*, **52**, 69–76.

Bishop, C.D., Erezyilmaz, D.F., Flatt, T., Georgiou, C.D., Hadfield, M.G., Heyland, A., Hodin, J., Jacobs, M.W., Maslakova, S.A., Pires, A., Reitzel, A.M., Santagata, S., Tanaka, K. & Youson, J.H. (2006) What is metamorphosis? *Integrative and Comparative Biology*, **46**, 655–661.

Blackstone, N.W. & Joslyn, A.R. (1984) Utilization and preference for the introduced gastropod *Littorina littorea* (L) by the hermit crab *Pagurus longicarpus* (Say)

at Guilford, Connecticut. *Journal of Experimental Marine Biology and Ecology*, **80**, 1–9.

Blaxter, J.H.S. (1986) Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. *Transactions of the American Fisheries Society*, **115**, 98–114.

Blaxter, J.H.S. & Hempel, G. (1963) The influence of egg size on herring larvae (*Clupea harengus* L.). *Journal du Conseil International pour l'Exploration de la Mer*, **28**, 211–240.

Bliss, D.E. & Boyer, J.R. (1964) Environmental regulation of growth in the decapod crustacean *Gecarcinus lateralis*. *General and Comparative Endocrinology*, **4**, 15–41.

Bolaños, J., Cuesta, J.A., Hernández, G., Hernández, J. & Felder, D.L. (2004) Abbreviated larval development of *Tunicotheres moseri* (Rathbun, 1918) (Decapoda, Pinnotheridae), a rare case of parental care in brachyuran crabs. *Scientia Marina*, **68**, 373–384.

Bolton, T.F. & Graham, W.M. (2006) Jellyfish on the rocks: bioinvasion threat of the international trade in aquarium live rock. *Biological Invasions*, **8**, 651–653.

Booth, J.D., Carruthers, A.D., Bolt, C.D., Stewart, R.A. (1991) Measuring depth of settlement in the red rock lobster, *Jasus edwardsii*. *New Zealand Journal of Marine and Freshwater Research*, **25**, 123–132.

Booth, J.D., Stotter, D.R., Forman, J.S. & Bradford, E. (2001) Juvenile abundance both mirrors and masks a settlement pulse of the rock lobster *Jasus edwardsii*. *Marine and Freshwater Research*, **52**, 1067–1075.

Booth, J.D., Webber, W.R., Sekiguchi, H. & Coutures, E. (2005) Diverse larval recruitment strategies within the Scyllaridae. *New Zealand Journal of Marine and Freshwater Research*, **39**, 581–592.

Botero, L. & Atema, J. (1982) Behavior and substrate selection during larval settling in the lobster *Homarus americanus*. *Journal of Crustacean Biology*, **2**, 59–69.

Boudreau, B., Bourget, E. & Simard, Y. (1990) Benthic invertebrate larval response to substrate characteristics at settlement: shelter preferences of the American lobster *Homarus americanus*. *Marine Biology*, **106**, 191–198.

Bourne, D.G., Young, N.S., Webster, N., Payne, M., Salmon, M., Demel, S. & Hall, M. (2004) Microbial community dynamics in a larval aquaculture system of the tropical rock lobster, *Panulirus ornatus*. *Aquaculture*, **242**, 31–51.

Bratvold, D. & Browdy, C.L. (2001) Effects of sand sediment and vertical surfaces (Aquamats) on production, water quality, and microbial ecology in an intensive *Litopenaeus vannamei* culture system. *Aquaculture*, **195**, 81–94.

Bray, W.A. & Lawrence, A.L. (1992) Reproduction of *Penaeus* species in captivity. In: *Marine Shrimp Culture: Principles and Practices* (eds A.W. Fast & J.L. Lester) pp. 93–170. Elsevier, Amsterdam, The Netherlands.

Bray, W.A., Lawrence, A.L. & Lester, L.J. (1990) Reproduction of eyestalk-ablated *Penaeus stylirostris* fed various levels of total dietary lipid. *Journal of the World Aquaculture Society*, **21**, 41–52.

Briggs, M.R.P. (1992) A stress test for determining vigour of post-larval *Penaeus monodon* Fabricius. *Aquaculture and Fisheries Management*, **23**, 633–637.

Brito, R., Rosas, C., Chimal, M. & Gaxiola, G. (2001) Effect of different diets on growth and digestive enzyme activity in *Litopenaeus vannamei* (Boone, 1931) early post larvae. *Aquaculture Research*, **32**, 257–266.

Broad, A.C. (1957) Larval development of the crustacean *Thor floridanus* Kingsley. *Journal of the Elisha Mitchell Scientific Society*, **73**, 317–328.

Brooks, W.K. & Herrick, F.H. (1893) The embryology and metamorphosis of the Macrura. *Memoirs of the National Academy of Sciences*, **5**, 339–351.

Browdy, C. (1992) A review of the reproductive biology of *Penaeus* species: perspectives on controlled shrimp maturation systems for high quality nauplii production. In: *Proceedings of the Special Session on Shrimp Farming, World Aquaculture Society Meeting* (ed J. Wyban) pp. 22–51. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Browdy, C.L. & Samocha, T.M. (1985) Maturation and spawning of ablated and nonablated *Penaeus semisulcatus* (De Haan, 1844). *Journal of the World Mariculture Society*, **16**, 236–249.

Brown, F.A. & Jones, G.M. (1949) Ovarian inhibition by a sinus-gland principle in the fiddler crab. *Biological Bulletin*, **96**, 228–232.

Brown, J.H. (1989) Antimicrobials: their use and abuse in aquaculture. *World Aquaculture*, **20**, 34–43.

Brown, M.R. (2002) Nutritional value of microalgae for aquaculture. In: *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola* (eds L.E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M.G. Gaxiola-Cortés & N. Simões) pp. 281–292. Cancún, Quintana Roo, México.

Brown, M.R., Jeffrey, S.W., Volkman J.K. & Dunstan, G.A (1997) Nutritional properties of microalgae for mariculture. *Aquaculture*, **151**, 315–331.

Bruce, A.J. (1975) Les crevettes des récifs coralliens et leurs colleurs. *Endeavour*, **24**, 23–27.

Bruce, A.J. (1976a) Shrimps and prawns of coral reefs, with special reference to commensalism. In: *Biology and Geology of Coral Reefs*, Vol. 3 (Biology 2) (eds O.A. Jones & R. Endean) pp. 37–94. Academic Press, New York, USA.

Bruce, A.J. (1976b) Coral reef Caridea and "commensalism". *Micronesia*, **12**, 83–98.

Bruce, A.J. (1986) Observations on the family Gnathophyllidae Dana, 1852 (Crustacea: Decapoda). *Journal of the Crustacean Society*, **6**, 463–470.

Brumbaugh, R.D. & McConaugha, J.R. (1995) Time to metamorphosis of the blue crab *Callinectes sapidus* megalopae: effects of benthic macroalgae. *Marine Ecology Progress Series*, **129**, 113–118.

Bshary, R. (2003) The cleaner wrasse, *Labroides dimidiatus*, is a key organism for reef fish diversity at Ras Mohammed National Park, Egypt. *Journal of Animal Ecology*, **72**, 169–176.

Buchan, K.A.H., Martin-Robichaud, D.J. & Benfey, T.J. (2005) Measurement of dissolved ozone in sea water: a comparison of methods. *Aquacultural Engineering*, **33**, 225–231.

Bullard, S.G., Lindquist, N. & Hay, M.E. (1999) Susceptibility of invertebrate larvae to predators: how common are post-capture larval defences? *Marine Ecology Progress Series*, **191**, 153–161.

Bundy, M.H. (1983) Simultaneous functional hermaphroditism in the shrimp *Hippolysmata wurdemanni* (Gibbes) (Decapoda: Caridea: Hippolytidae). MS thesis, Old Dominion University, Norfolk, VA.

Bunkley-Williams, L. & Williams, E.H. (1998) Ability of Pederson cleaner shrimp to remove juveniles of the parasitic cymothoid isopod, *Anilocra haemuli*, from the host. *Crustaceana*, **71**, 862–869.

Bunting, B., Holthus, P. & Spalding, S. (2003) The marine aquarium industry and reef conservation. In: *Marine Ornamental Species: Collection, Culture and Conservation* (eds J.C. Cato & C.L. Brown) pp. 109–124. Iowa State Press, Ames, Iowa, USA.

Burke, R.D. (1983) The induction of metamorphosis of marine invertebrate larvae: stimulus and response. *Canadian Journal of Zoology*, **61**, 1701–1719.

Burrows, M. (1969) The mechanics and neural control of the prey capture strike in the mantid shrimps *Squilla* and *Hemisquilla*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, **62**, 361–381.

Burukovski, R.N. (2000) *Lysmata splendida* sp. nov., a new species of shrimp from the Maldives (Crustacea: Decapoda: Hippolytidae). *Senckenbergiana maritima*, **30**, 223–227.

Butler, IV, M.J. & Herrnkind, W.F. (1991) Effect of benthic micro-habitat cues on the metamorphosis of pueruli of the spiny lobster *Panulirus argus*. *Journal of Crustacean Biology*, **11**, 23–28.

Cahu, C.L., Guillaume, J.C., Stéphan, G. & Chim, L. (1994) Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg tissue composition in *Penaeus vannamei* fed semi-purified diets. *Aquaculture*, **126**, 159–170.

Cahu, C.L., Cuzan, G. & Quazuguel, P. (1995) Effect of highly unsaturated fatty acids, alpha-tocopherol and ascorbic acid in broodstock diet on egg composition and development of *Penaeus indicus*. *Comparative Biochemistry and Physiology*, **112**, 417–424.

Calado, R. (2005) Avaliação do potencial de cultivo do camarão ornamental *Lysmata seticaudata* (Risso, 1816) (Decapoda: Hippolytidae). PhD thesis, Universidade de Lisboa, Lisboa, Portugal.

Calado, R. (2006) Marine ornamental species from European waters: a valuable overlooked resource or a future threat for the conservation of marine ecosystems? *Scientia Marina*, **70**, 389–398.

Calado, R. & Chapman, P.M. (2006) Aquarium species: deadly invaders. *Marine Pollution Bulletin*, **52**, 599–601.

Calado, R. & Dinis, M.T. (2007) Minimization of precocious sexual phase change during culture of juvenile ornamental shrimps *Lysmata seticaudata* (Decapoda: Hippolytidae). *Aquaculture*, **269**, 299–305.

Calado, R. & Dinis, M.T. (in press) Collection of marine invertebrates for the aquarium trade in European waters: is anyone surveying? *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Calado, R. & Narciso, L. (2003a) Seasonal variation on embryo production and brood loss in the Monaco shrimp *Lysmata seticaudata* (Decapoda: Hippolytidae). *Journal of the Marine Biological Association of the United Kingdom*, **83**, 959–962.

Calado, R. &. Narciso, L. (2003b) Can all ornamental decapods be raised in captivity? Pros and cons of profitability in conservation. *World Aquaculture 2003. Salvador, Brazil. Book of Abstracts*, 145.

Calado, R. & Narciso, L. (2005) Ability of Monaco shrimp *Lysmata seticaudata* (Decapoda: Hippolytidae) to control the pest glass anemone *Aiptasia pallida* (Actiniaria: Aiptasidae). *Helgoland Marine Research*, **59**, 163–165.

Calado, R., Martins, C., Santos, O. & Narciso, L. (2001a) Larval development of the Mediterranean cleaner shrimp *Lysmata seticaudata* (Risso, 1816) (Caridea: Hippolytidae) fed on different diets — costs and benefits of mark-time molting. *European Aquaculture Society, Special Publication*, **30**, 96–99.

Calado, R., Morais, S. & Narciso, L. (2001b) Temperate shrimps: perspective use as ornamental species. *Marine Ornamentals 2001. Collection, Culture and Conservation. Orlando, Florida, USA. Book of Abstracts*, 118–119.

Calado, R., Narciso, L., Morais, S., Rhyne, A.L. & Lin, J. (2003a) A rearing system for the culture of ornamental decapod crustacean larvae. *Aquaculture*, **218**, 329–339.

Calado, R., Narciso, L., Araújo, R. & Lin, J. (2003b) Overview of marine ornamental shrimp aquaculture. In: *Marine Ornamental Species: Collection, Culture and Conservation* (eds J.C. Cato & C.L. Brown) pp. 221–230. Iowa State Press, Ames, Iowa, USA.

Calado, R., Lin, J., Rhyne, A.L., Araújo, R. & Narciso, L. (2003c) Marine ornamental decapods — popular, pricey, and poorly studied. *Journal of Crustacean Biology*, **23,** 963–973.

Calado, R., Bartilotti, C., Narciso, L. & Dos Santos, A. (2004) Redescription of the larval stages of *Lysmata seticaudata* (Risso, 1816) (Crustacea, Decapoda, Hippolytidae) reared under laboratory conditions. *Journal of Plankton Research*, **26**, 737–752.

Calado, R., Figueiredo, J., Rosa, R., Nunes, M.L. & Narciso, L. (2005a) Larval culture of Monaco shrimp *Lysmata seticaudata* (Decapoda: Hippolytidae): effect of temperature, rearing density and larval diet. *Aquaculture*, **245**, 221–237.

Calado, R., Conceição, L.E. & Dinis, M.T. (2005b) Early larval culture of marine ornamental decapods: getting it right from the start. *European Aquaculture Society Special Publication*, **36**, 67–70.

Calado, R., Bartilotti, C. & Narciso, L. (2005c) Short report on the effect of a parasitic isopod on the reproductive performance of a shrimp. *Journal of Experimental Marine Biology and Ecology*, **321**, 13–18.

Calado, R., Rosa, R., Nunes, M.L. & Narciso, L. (2005d) Amino and fatty acid dynamics of *Lysmata seticaudata* (Decapoda: Hippolytidae) embryos during early and late reproductive season. *Marine Biology*, **147**, 341–351.

Calado, R., Rosa, R., Morais, S., Nunes, M.L. & Narciso, L. (2005e) Growth, survival and lipid profile of juvenile Monaco shrimp *Lysmata seticaudata* fed on different diets. *Aquaculture Research*, **36**, 493–504.

Calado, R., Vitorino, A. & Dinis, M.T. (2006) Bopyrid isopods do not castrate the simultaneous hermaphroditic shrimp *Lysmata amboinensis* (Decapoda: Hippolytidae). *Diseases of Aquatic Organisms*, **73**, 73–76.

Calado, R., Dionísio, G. & Dinis, M.T. (2007a) Decapod crustaceans associated with anemone *Anemonia sulcata* — living there or just passing by? *Scientia Marina*, **71**, 287–292.

Calado, R., Dionísio, G., Nunes, C. & Dinis, M.T. (2007b) Facultative secondary lecithotrophy in the megalopa of the shrimp *Lysmata seticaudata* (Risso, 1816) (Decapoda: Hippolytidae) under laboratory conditions. *Journal of Plankton Research*, **29**, 599–603.

Calado, R., Dionísio, G. & Dinis, M.T. (2007c) Starvation resistance of early zoeal stages of marine ornamental shrimps *Lysmata* spp. (Decapoda: Hippolytidae) from different habitats. *Journal of Experimental Marine Biology and Ecology*, **351**, 226–233.

Calado, R., Vitorino, A., Dionísio, G. & Dinis, M.T. (2007d) A recirculated maturation system for marine ornamental decapods. *Aquaculture*, **263**, 68–74.

Caldwell, R.L. & Dingle, H. (1975) Ecology and evolution of agonistic behavior in stomatopods. *Naturwissenschaften*, **62**, 214–222.

Caldwell, R.L. & Dingle, H. (1976) Stomatopods. *Scientific American*, **234**, 80–89.

Calfo, A. (2007) *Book of Coral Propagation* (2nd Edn). Reading Trees Publications, Monroeville, Pennsylvania, USA.

Cameron, J.N. (1985) Molting in the blue crab. *Scientific American*, **252**, 102–109.

Cannicci, S., Garcia, L. & Galil, B.S. (2006) Racing across the Mediterranean — first record of *Percnon gibbesi* (Crustacea: Decapoda: Grapsidae) in Greece. *Journal of the Marine Biological Association of the United Kingdom 2 — Biodiversity Records*. Published online.

Carlisle, D.B. (1953) Studies on *Lysmata seticaudata* Risso (Crustacea Decapoda) V — The ovarian inhibiting hormone and the hormonal inhibition on sex-reversal. *Pubblicazione de la Stazione Zoologica di Napoli*, **24**, 355–372.

Carlisle, D.B. (1962) Some uncritical observations on reproductive behavior in crabs and prawns. *Animal Behaviour*, **10**, 383.

Carr, M.H. & Hixon, M.A. (1995) Predation effects on early post-settlement survivorship of coral reef fishes. *Marine Ecology Progress Series*, **124**, 31–42.

Carr, W.E.S., Netherton, J.C. & Milstead, M.L. (1984) Chemoattractants of the shrimp, *Palaemonetes pugio*: variability in responsiveness and the stimulatory capacity of mixtures containing amino acids, quaternary ammonium compounds, purines and other substances. *Comparative Biochemistry and Physiology, Part A*, **77**, 469–474.

Castille, F.L. & Lawrence, A.L. (1989) Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimps *Penaeus aztecus* Ives and *Penaeus setiferus* (L.). *Journal of Crustacean Biology*, **9**, 202–211.

Castille, F.L., Samocha, T.M., Lawrence, A.L., He, H., Frelier, P. & Jaenike, F. (1993) Variability in growth and survival of early postlarval shrimp (*Penaeus vannamei* Boone 1931). *Aquaculture*, **113**, 65–81.

Castro, P. (1976) Brachyuran crabs symbiotic with scleractinian corals: a review of their biology. *Micronesica*, **12**, 99–110.

Castro, P., Ng, P.K.L. & Ahyong, S.T. (2004) Phylogeny and systematics of the Trapeziidae Miers, 1886 (Crustacea: Brachyura), with the description of a new family. *Zootaxa*, **643**, 1–70.

Catacutan, M.R. (2002) Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. *Aquaculture*, **208**, 113–123.

Cavalli, R.O., Scardua, M.P. & Wasielesky, W.J. (1997) Reproductive performance of different-sized wild and pond-reared *Penaeus paulensis* females. *Journal of the World Aquaculture Society*, **28**, 260–267.

Cavalli, R.O., Lavens, P. & Sorgeloos, P. (1999) Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. *Aquaculture*, **179**, 387–402.

Cavalli, R.O., Menschaert, G., Lavens, P. & Sorgeloos, P. (2000a) Maturation performance, offspring quality and lipid composition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. *Aquaculture International*, **8**, 41–58.

Cavalli, R.O., Van den Berghe, E., Lavens, P., Thuy, T.T.N., Wille, M. & Sorgeloos, P. (2000b) Ammonia toxicity as a criterion for the evaluation of larval quality in the prawn *Macrobrachium rosenbergii*. *Comparative Biochemistry and Physiology, Part C*, **125**, 333–343.

Cevik, C., Yokes, M.B., Cavas, L., Erkol, L.I., Derici, O.B. & Verlaque, M. (2007) First report of *Caulerpa taxifolia* (Bryopsidales, Chlorophyta) on the Levantine coast (Turkey, Eastern Mediterranean). *Estuarine, Coastal and Shelf Science*, **74**, 549–556.

Chace, F.A., Jr (1997) The caridean shrimps (Crustacea: Decapoda) of the Albatross Philippine Expedition, 1907–1910, Part 7: Families Atyidae, Eugonatonotidae, Rhynchocinetidae, Bathypalaemonellidae, Processidae, and Hippolytidae. *Smithsonian Contributions to Zoology*, **587**, 1–106.

Charmantier, G. & Charmantier-Daures, M. (2001) Ontogeny of osmoregulation in crustaceans: the embryonic phase. *American Zoologist*, **41**, 1078–1089.

Charniaux-Cotton, H. (1958) La glande androgéne de quelques Crustacés Décapodes et particuliérement de *Lysmata seticaudata*, espéce á hermaphrodisme protérandrique fonctionnel. *Comptes Rendus des Séances de la Société de Biologie*, **246**, 2814–2817.

Charniaux-Cotton, H. (1959a) Masculinisation des femelles de la crevette á hermaphrodisme protérandrique *Lysmata seticaudata*, par greffe de glandes androgénes. Interprétation de l'hermaphrodisme chez les Décapodes. Note preliminaire. *Comptes Rendus des Séances de la Société de Biologie*, **249**, 1580–1582.

Charniaux-Cotton, H. (1959b) Physiologie de la gonade de *Lysmata seticaudata* (crevette à hermaphrodisme protérandrique) chez les individus normaux et les femelles masculinisées. *Comptes Rendus des Séances de la Société de Biologie*, **252**, 199–201.

Charniaux-Cotton, H. (1960) Physiologie de l'inversion sexuelle chez la crevette à hermaphrodisme protérandrique fonctionnel, *Lysmata seticaudata*. *Comptes Rendus des Séances de la Société de Biologie*, **250**, 4046–4048.

Charniaux-Cotton, H. (1961) Physiologie de la gonade de *Lysmata seticaudata* (crevette à hermaphrodisme protérandrique) chez les individus normaux et les femelles masculinisées. *Comptes Rendus des Séances de la Société de Biologie*, **252**, 199–201.

Charniaux-Cotton, H. (1985) Vitellogenesis and its control in malacostracan Crustacea. *American Zoologist*, **25**, 197–206.

Charniaux-Cotton, H. & Payen, G. (1988) Crustacean reproduction. In: *Endocrinology of Selected Invertebrate Types* (eds H. Laufer & R.G.H. Downer) pp. 279–303. Alan R. Liss, New York, USA.

Charniaux-Cotton, H. & Touir, A. (1973) Contrôle de la prévitellogenése et de la vitellogenése chez la crevette hermaphrodite *Lysmata seticaudata* Risso. *Compte Rendu des Seances de l'Academie des Sciences*, **276**, 2717–2720.

Chassard-Bouchaud, C. (1965) L'ádaptation chromatique chez les Natantia (Crustacés Décapodes). *Cahiers de Biologie Marine*, **6**, 469–576.

Chassard-Bouchaud, C. & Couturier, Y. (1968) Étude des phénomènes chromatiques de *Lysmata seticaudata* Risso (Crustacé Décapode). I — Livrée chromatique et cycle nycthéméral. *Cahiers de Biologie Marine*, **9**, 201–209.

Chassard-Bouchaud, C. & Couturier, Y. (1969) Étude des phénomènes chromatiques de *Lysmata seticaudata* Risso (Crustacé Décapode). II — Effets de l'ablation des pédoncules oculaires sur le cycle nycthéméral. *Cahiers de Biologie Marine*, **10**, 173–180.

Chatterjee, L.B. (1973) Evolution and biosynthesis of ascorbic acid. *Science*, **182**, 1271–1272.

Chen, H.-Y. (1998) Nutritional requirements of the black tiger shrimp: *Penaeus monodon*. *Reviews in Fisheries Science*, **6**, 79–95.

Chockley, B.R. & St. Mary, C.M. (2003) Effects of body size on growth, survivorship, and reproduction in the banded coral shrimp *Stenopus hispidus*. *Journal of Crustacean Biology*, **23**, 836–848.

Christy, J.H. (1989) Rapid development of megalopae of the fiddler crab *Uca pugilator* reared over sediment: implications for models of larval recruitment. *Marine Ecology Progress Series*, **57**, 259–265.

Clark, P.F., Calazans, D. & Pohle, W. (1998) Accuracy and standardization of brachyuran larval descriptions. *Invertebrate Reproduction and Development*, **33**, 127–144.

Clarke, A. (1982) Lipid synthesis and reproduction in the polar shrimp *Chorismus antarcticus*. *Marine Ecology Progress Series*, **9**, 81–90.

Clarke, A. (1993) Egg size and egg composition in polar shrimps (Caridea; Decapoda). *Journal of Experimental Marine Biology and Ecology*, **168**, 189–203.

Clemmesen, C. (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology*, **118**, 377–382.

Cobo, V.J. (2002) Breeding period of the arrow crab *Stenorhynchus seticornis* from Couves Island, south-eastern Brazilian coast. *Journal of the Marine Biological Association of the United Kingdom*, **82**, 1031–1032.

Coen, L.D. (1988a) Herbivory by Caribbean majid crabs: feeding ecology and plant susceptibility. *Journal of Experimental Marine Biology and Ecology*, **122**, 257–276.

Coen, L.D. (1988b) Herbivory by crabs and the control of algal epibionts on Caribbean host corals. *Oecologia*, **75**, 198–203.

Coeuret, V., Guwguen, M. & Vernoux, J.P. (2004) Numbers and strain of lactobacilli in some probiotic products. *International Journal of Food Microbiology*, **97**, 147–156.

Cohen, J.H. & Forward, R.B., Jr (2003) Ctenophore kairomones and modified aminosugar disaccharides alter the shadow response in a larval crab. *Journal of Plankton Research*, **25**, 203–213.

Cole, B., Tamaru, C.S., Bailey, R., Brown, C. & Ako, H. (1999) Shipping practices in the ornamental fish industry. *Center for Tropical and Subtropical Aquaculture, Publication Number 131*. Waimanalo, Hawaii, USA.

Coman, G.J. & Crocos, P.J. (2003) Effect of age on the consecutive spawning of ablated *Penaeus semisulcatus* broodstock. *Aquaculture*, **219**, 445–456.

Coman, G.J., Sarac, H.Z., Fielder, D. & Thorne, M. (1996) Evaluation of crystalline amino acids, betaine and AMP as food attractants of the giant tiger prawn (*Penaeus monodon*). *Comparative Biochemistry and Physiology, Part A*, **113**, 247–253.

Coman, G.J., Sellars, M.J. & Morehead, D.T. (2005) Toxicity of ozone generated from different combinations of ozone concentration (C) and exposure time (T): a comparison of the relative effect of C and T on hatch rates of *Penaeus* (*Marsupenaeus*) *japonicus*. *Aquaculture*, **244**, 141–145.

Coman, G.J., Arnold, S.J., Callaghan, T.R. & Preston, N.P. (2007) Effect of two maturation diet combinations on reproductive performance of domesticated *Penaeus monodon*. *Aquaculture*, **263**, 75–83.

Conklin, D.E. (1997) Vitamins. In: *Advances in World Aquaculture*, Vol. 6 (Crustacean Nutrition) (eds L.R. D'Abramo, D.E. Conklin & D.M. Akiyama) pp. 123–149. The World Aquaculture Society, Baton Rouge, Louisiana, USA.

Contreras-Garduño, J. & Córdoba-Aguilar, A. (2006) Sexual selection in hermit crabs: a review and outlines of future research. *Journal of Zoology, London*, **270**, 595–605.

Corey, S. & Reid, D. M. (1991) Comparative fecundity of decapod crustaceans I. The fecundity of thirty-three species of nine families of Caridean shrimps. *Crustaceana*, **60**, 270–294.

Correa, C. & Thiel, M. (2003) Mating systems in caridean shrimp (Decapoda: Caridea) and their evolutionary consequences for sexual dimorphism and reproductive biology. *Revista Chilena de História Natural*, **76**, 187–203.

Correa, C., Baeza, J., Dupré, E., Hinojosa, I. & Thiel, M. (2000) Mating behavior and fertilization success of three ontogenetic stages of male rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea). *Journal of Crustacean Biology*, **20**, 628–640.

Correa, C., Baeza, J.A., Hinojosa, I.A. & Thiel, M. (2003) Dominance hierarchy and mating tactics in the rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea). *Journal of Crustacean Biology*, **23**, 33–45.

Courtenay, W.R. & Stauffer, J.R. (1990) The introduced fish problem and the aquarium fish industry. *Journal of the World Aquaculture Society*, **21**, 145–159.

Costa-Pierce, B.A. & Laws, E.A. (1985) Chemotactically-active feed additive for shrimps (*Macrobrachium rosenbergii*). *The Progressive Fish Culturist*, **47**, 59–61.

Côté, I.M. (2000) Evolution and ecology of cleaning symbioses in the sea. *Oceanography and Marine Biology: an Annual Review*, **38**, 311–355.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P. & Sorgeloos, P. (1997) Review on the dietary effects of phospholipids in fish and crustacean larviculture. *Aquaculture*, **155**, 149–164.

Couturier-Bhaud, Y. (1974) Cycle biologique de *Lysmata seticaudata* Risso (Crustacé, Décapode). III — Étude du développement larvaire. *Vie et Millieu*, **24**, 431–442.

Cowey, C.B. & Forster, J.R.M. (1971) The essential amino acid requirements of prawn, *Palaemon serratus*. *Marine Biology*, **10**, 77–81.

Cox, S. & Johnston, D. (2003a) Feeding biology of spiny lobster larvae and implications for culture. *Reviews in Fisheries Science*, **11**, 89–106.

Cox, S. & Johnston, D. (2003b) Developmental changes in the structure and function of mouthparts of phyllosoma larvae of the packhorse lobster, *Jasus verreauxi* (Decapoda: Palinuridae). *Journal of Experimental Marine Biology and Ecology*, **296**, 35–47.

Crisp, D.J. (1974) Factors influencing the settlement of marine invertebrate larvae. In: *Chemoreception in Marine Organisms* (eds P.T. Grant & A.M. Mackie) pp. 177–265. Academic Press, London, UK.

Crocos, P.J. & Coman, G.J. (1997) Seasonal and age variability in the reproductive performance of *Penaeus semisulcatus*: optimizing broodstock selection. *Aquaculture*, **155**, 55–67.

Crompton, W.D. (1992) Laboratory culture and larval development of the peppermint shrimp, *Lysmata wurdemanni* (Gibbes) (Caridea: Hippolytidae). MS thesis, Corpus Christi State University, Corpus Christi, Texas, USA.

Cronin, T.W. (1986) Photoreception in marine invertebrates. *American Zoologist*, **26**, 403–415.

Cruz-Suarez, L.E., Ricque-Marie, D., Pinal-Mansilla, J.D. & Wesche-Ebelling, P. (1994) Effect of different carbohydrate sources on the growth of *Penaeus vannamei*: economic impact. *Aquaculture*, **123**, 349–360.

Cuesta, J.A. & Rodríguez, A. (2000) Zoeal stages of the intertidal crab *Pachygrapsus marmoratus* (Fabricius, 1787) (Brachyura, Grapsidae) reared in the laboratory. *Hydrobiologia*, **436**, 119–130.

Curtis, M.C. & Jones, C.M. (1995) Observations on monosex culture of redclaw crayfish *Cherax quadricarinatus* von Martens (Decapoda: Parastacidae) in earthen ponds. *Journal of the World Aquaculture Society*, **26**, 154–159.

Cushing, B.S. & Reese, E. (1988) Hawk-like aggression in the Hawaiian red lobster, *Enoplometopus occidentalis*. *Behaviour*, **135**, 863–877.

Cutts, C. J. (2003) Culture of harpacticoid copepods: potential as live feed for rearing marine fish. *Advances in Marine Biology*, **44**, 295–316.

Cuzon, G., Rosas, C., Gaxiola, G., Taboada, G. & Van Wormhoudt, A. (2001) Effect of dietary carbohydrates on gluconeogenesis in premoult *Litopenaeus stylirostris* juveniles and pre-adults. *Journal of Shellfish Research*, **20**, 931–937.

D'Abramo, L.R. (1989) Lipid requirements of shrimp. Advances in Tropical Aquaculture. *AQUACOPIFREMER, Tahiti*, 271–285.

D'Abramo, L.R. (1997) Triacylglycerol and fatty acids. In: *Crustacean Nutrition*, Vol. 6 (ed. E.Ž. Halver) pp. 71–84. World Aquaculture Society, Baton Rouge, Louisiana, USA.

D'Abramo, L.R. (1998) Nutritional requirements of the freshwater prawn *Macrobrachium rosenbergii*: comparisons with species of penaeid shrimp. *Reviews in Fisheries Science*, **6**, 153–163.

D'Abramo, L.R. (2002) Challenges in developing successful formulated feed for culture of larval fish and crustaceans. In: *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola*. (eds L.E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M.G. Gaxiola-Cortés & N. Simões) pp. 143–151. Cancún, Quintana Roo, México.

Dale, W.E. & Anderson, G. (1982) Comparison of morphologies of *Probopyrus bithynis*, *P. floridensis*, and *P. pandalicola* larvae reared in culture (Isopoda, Epicaridea). *Journal of Crustacean Biology*, **2**, 392–409.

Dall, W. (1995) Carotenoids versus retinoids (vitamin A) as essential growth factors in penaeid prawns (*Penaeus semisulcatus*). *Marine Biology*, **124**, 209–213.

Dall, W., Hill, B.J., Rothlisberg, P.C. & Staples, D.J. (1990) The biology of the Penaeidae. *Advances in Marine Biology*, **27**, 1–467.

Dall, W., Smith, D.M. & Moore, L.E. (1995) Carotenoids in the tiger prawn *Penaeus esculentus* during ovarian maturation. *Marine Biology*, **123**, 435–441.

Danald, D., Ure, J. & Lightner, D. (1979) Preliminary results of ozone disinfection of seawater containing the potential shrimp pathogens *Vibrio* sp. and *Fusarium solani*. *Ozone: Science and Engineering*, **1**, 329–334.

Daniel, P.C. & Bayer, R.C. (1987) Temporal changes in release rates and quality of lobster (*Homarus americanus*) feeding attractants from herring (*Clupea harengus*) baits. *Marine Behavior and Physiology*, **13**, 13–27.

Daniel, P.C. & Bayer, R.C. (1989) Fish byproducts as chemoattractant substrates for the American lobster (*Homarus americanus*): concentration, quality and release characteristics. *Fisheries Research*, **7**, 367–383.

Daniel, P.C. & Derby, C.D. (1991) Chemosensory response to mixtures: a model based on composition of receptor cell types. *Physiology and Behavior*, **49**, 581–589.

Davis, D., Lawrence, A. & Gatlin III, D. (1992) Mineral requirements of *Penaeus vannamei*: a preliminary examination of the dietary essentiality for thirteen minerals. *Journal of the World Aquaculture Society*, **23**, 8–14.

Davis, D.A., Arnold, C.R. & McCallum, I. (2002) Nutritional value of feed peas (*Pisum sativum*) in practical diet formulations for *Litopenaeus vannamei*. *Aquaculture Nutrition*, **8**, 87–94.

Day, EA. & Lawton, P. (1988) Mud crab (Crustacea: Brachyura: Xanthidae) substrate preference and activity. *Journal of Shellfish Research*, **7**, 421–426.

De'ath, G. & Moran, P.J. (1998a) Factors affecting the behaviour of crown-of-thorns starfish (*Acanthaster planci* L.) on the Great Barrier Reef: 1: Patterns of activity. *Journal of Experimental Marine Biology and Ecology*, **220**, 83–106.

De'ath, G. & Moran, P. J. (1998b) Factors affecting the behaviour of crown-of-thorns starfish (*Acanthaster planci* L.) on the Great Barrier Reef: 2: Feeding preferences. *Journal of Experimental Marine Biology and Ecology*, **220**, 107–126.

Debelius, H. (1984) *Armoured Knights of the Sea*. Quality Marine, Los Angeles, California, USA.

Debelius, H. (2001) *Crustacea Guide of the World*. IKAN — Unterwasserarchive, Frankfurt, Germany.

Delbeeck, J.C. & Sprung, J. (2005) *The Reef Aquarium, Vol. 3, Science, Art, and Technology*. Ricordea Publishing, Coconut Grove, Florida, USA.

Derby, C.D. (2000) Learning from spiny lobsters about chemosensory coding of mixtures. *Physiology and Behavior*, **69**, 203–209.

Descouturelle, G. (1971) Rôles des appendices sexuels mâles d'un la copulation et d'un réceptacle séminal femelle dans la ponte chez *Atyaephyra desmaresti* Millet (Crustacea–Decapoda–Caridea). *Bulletin de l'Académie et de la Société Lorraines des Sciences*, **10**, 10–14.

Deshimaru, O. (1982) Protein and amino acid nutrition of the prawn, *Penaeus japonicus*. *Proceedings of the 2nd International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. World Mariculture Society, Special Publication*, **2**, 106–122.

Deshimaru, O. & Yone, Y. (1978) Effect of dietary carbohydrate sources on the growth and feed efficiency of prawn. *Bulletin of the Japanese Society of Scientific Fisheries*, **44**, 1161–1163.

Deudero, S., Frau, A., Cerda, M. & Hampel, H. (2005) Distribution and densities of the decapod crab *Percnon gibbesi*, an invasive Grapsidae, in western Mediterranean waters. *Marine Ecology Progress Series*, **285**, 151–156.

Dhert, P., Lavens, P. & Sorgeloos, P. (1992) Stress evaluation: a tool for quality control of hatchery-produced shrimp and fish fry. *Aquaculture Europe*, **17**, 6–10.

Dhert, P., Rombaut, G., Suantika, G. & Sorgeloos, P. (1997) Advancement of rotifer culture and manipulation techniques in Europe. *Aquaculture*, **200**, 129–146.

Dhont, J. & Van Stappen, G. (2003) Biology, tank production and nutritional value of *Artemia franciscana*. In: *Live Feeds in Marine Aquaculture* (eds J.G. Stottrup & L.A. McEvoy) pp. 65–121. Blackwell Science, Oxford, UK.

Diesel, R. (1988) Discrete storage of multiple-mating sperm in the spider crab *Inachus phalangium*. *Naturwissenschaften*, **75**, 148–149.

Dittel, A.I., Hines, A.H., Ruiz, G.M. & Ruffin, K.K.E. (1995) Effects of shallow water refuge on behavior and density-dependent mortality of juvenile blue crabs in Chesapeake Bay. *Bulletin of Marine Sciences*, **57**, 902–916.

Djunaidah, I.S., Wille, M., Kontara, E.K. & Sorgeloos, P. (2003) Reproductive performance and offspring quality in mud crab (*Scylla paramamosain*) broodstock fed different diets. *Aquaculture International*, **11**, 3–15.

Dobkin, S. (1968) The larval development of a species of *Thor* (Caridea, Hippolytidae) from South Florida, USA. *Crustaceana*, Supplement 2, 1–18.

Doherty, P.J. (1987) Light traps: selective but useful devices for quantifying the distributions and abundances of larval fishes. *Bulletin of Marine Science*, **41**, 423–431.

Doherty, P.J., Dufour, V., Galzin, R., Hixon, M.A., Meekan, M.G. & Planes, S. (2004) High mortality during settlement is a population bottleneck for a tropical surgeonfish. *Ecology*, **85**, 2422–2428.

Dohrn, P.F.R. (1950) Studi sulla *Lysmata seticaudata* Risso (Hippolytidae). I — Le condizioni normali della sessualitá in natura. *Pubblicazione de la Stazione Zoologica di Napoli*, **22**, 257–272.

Dos Santos, A. & González-Gordillo, I. (2004) Illustrated keys for the identification of the Pelocyemata (Crustacea: Decapoda) zoeal stages, from the coastal region

of south-western Europe. *Journal of the Marine Biological Association of the United Kingdom*, **84**, 205–227.

Dos Santos, A., Calado, R., Bartilotti, C. & Narciso, L. (2004) The larval development of the partner shrimp *Periclimenes sagittifer* (Norman, 1861) (Decapoda: Caridea: Palaemonidae: Pontoniinae) described from laboratory-reared material, with a note on chemical settlement cues. *Helgoland Marine Research*, **58**, 129–139.

Doupé, R.G. & Lymbery, A J. (2000) Managing translocations of aquatic species. *Aquaculture Research*, **31**, 151–156.

D'Souza, F.M.L., Lecossois, D., Heasman, M.P., Diemar, J.A., Jackson, C.J. & Pendrey, R.C. (2000) Evaluation of centrifuged microalgae concentrates as diets for *Penaeus monodon* Fabricius larvae. *Aquaculture Research*, **31**, 661–670.

D'Souza, F.M.L., Knuckey, R.M., Hohmann, S. & Pendrey, R.C. (2002) Flocculated microalgae concentrates as diets for larvae of the tiger prawn *Penaeus monodon* Fabricius. *Aquaculture Nutrition*, **8**, 113–120.

Duerden, J.E. (1905) On the habits and reactions of crabs bearing actinians in their claws. *Proceedings of the Zoological Society of London*, **2**, 494–511.

Duffy, J.E. (1996) Eusociality in coral reef shrimp. *Nature*, **381**, 512–514.

Duffy, J.E. (2002) The ecology and evolution of eusociality in sponge-dwelling shrimp. In: *Genes, Behavior, and Evolution in Social Insects* (ed. T. Kikuchi) pp. 1–38. University of Hokkaido Press, Sapporo, Japan.

Dufour, V. & Galzin, R. (1993) Colonization patterns of reef fish larvae to the lagoon at Moorea Island, French Polynesia. *Marine Ecology Progress Series*, **102**, 143–152.

Durville, P., Bosc, P., Galzin, R. & Conand, C. (2003) Aquacultural suitability of post-larval coral reef fish. *Secretariat of the Pacific Community Live Reef Fish Information Bulletin*, **11**, 18–30.

Eding, E.H., Kamstra, A., Verreth, J.A.J., Huisman, E.A. & Klapwijk, A. (2006) Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. *Aquacultural Engineering*, **34**, 234–260.

Ellis, S.C. & Ellis, E. (2002) Recent advances in lagoon-based farming practices for eight species of commercially valuable hard and soft corals — a technical report. *Center for Tropical and Subtropical Aquaculture, Publication Number 147*. Waimanalo, Hawaii, USA.

Ellis, S.C. & Sharron, L. (1999) The culture of soft corals (order: Alcyonacea) for the marine aquarium trade. *Center for Tropical and Subtropical Aquaculture, Publication Number 137*. Waimanalo, Hawaii, USA.

Elner, R.W. & Beninger, P.G. (1995) Multiple reproductive strategies in snow crab, *Chionoecetes opilio*: physiological pathways and behavioral plasticity. *Journal of Experimental Marine Biology and Ecology*, **193**, 92–112.

Endler, J.A. (1978) A predator's view of animal color patterns. *Evolutionary Biology*, **11**, 319–364.

Epelbaum, A. & Borisov, R. (2006) Feeding behaviour and functional morphology of the feeding appendages of red king crab *Paralithodes camtschaticus* larvae. *Marine Biology Research*, **2**, 77–88.

Epifanio, C.E., Cope, J.S., Rowe, P.M. & Jenkins, F.M. (1991) Comparison of rates of development of Atlantic mud crab larvae in the laboratory and in field-deployed enclosures. *Journal of Crustacean Biology*, **11**, 540–545.

Epifanio, C.E., Lobanoff, M.A., Connaughton, V.P. & Welch, J.M. (1994) Growth and development of Atlantic mud crab larvae fed natural zooplankton prey. *Journal of Experimental Marine Biology and Ecology*, **180**, 165–174.

Escobal, P.R. (2000) *Aquatic Systems Engineering: Devices and How They Function*, 2nd edn. Dimension Engineering Press, Oxnard, California, USA.

Farrow, G.E. (1971) Back reef and lagoonal environments of Aldabra Atoll distinguished by their crustacean burrows. *Symposia of the Zoological Society of London*, **28**, 455–500.

Farzanfar, A. (2006) The use of probiotics in shrimp aquaculture. *FEMS Immunology & Medical Microbiology*, **48**, 149–158.

Fast, A.W. & Menasveta, P. (2000) Some recent issues and innovations in marine shrimp pond culture. *Reviews in Fisheries Science*, **8**, 151–233.

Fautin, D.G., Guo, C.-G. & Hwang, J.-S. (1995) Costs and benefits of the symbiosis between *Periclimenes brevicarpalis* and its host *Entacmaea quadricolor*. *Marine Ecology Progress Series*, **129**, 77–84.

Felder, D.L., Martin, J.W. & Goy, J.W. (1985) Patterns in early postlarval development of decapods. In: *Crustacean Issues*, Vol. 2 (Larval Growth) (ed. A.M. Wenner) pp. 163–225. A.A. Balkema, Rotterdam, The Netherlands.

Felgenhauer, B.E. (1992) External anatomy and integumentary structures. In: *Microscopic Anatomy of Invertebrates*, Vol. 10 (Decapod Crustacea) (eds F.W. Harrison & A.G. Humes) pp. 7–43. Wiley-Liss, New York, USA.

Ferezou, J.P., Barbier, M. & Berreur-Bonnenfant, J. (1978) Biosynthese de la farnesylacetone-(E-E) par les glandes androgens du crabe *Carcinus maenas*. *Helvetica Chimica Acta*, **61**, 669–674.

Fernández-Reiriz, M.J. & Labarta, U. (1996) Lipid classes and fatty acid composition of rotifers (*Brachionus plicatilis*) fed two algal diets. *Hydrobiologia*, **330**, 73–79.

Fiedler, G.C. (1994) *The larval stages of the harlequin shrimp* Hymenocera picta *(Dana)*. MS thesis, University of Hawaii, Honolulu.

Fiedler, G.C. (1998) Functional, simultaneous hermaphroditism in female-phase *Lysmata amboinensis* (Decapoda: Caridea: Hippolytidae). *Pacific Science*, **52**, 161–169.

Fiedler, G.C. (2000) *Sex determination and reproductive biology of two caridean shrimp genera:* Hymenocera *and* Lysmata. PhD thesis, University of Hawaii.

Fiore, D.R. & Tlusty, M.F. (2005) Use of commercial *Artemia* replacement diets in culturing larval American lobsters (*Homarus americanus*). *Aquaculture*, **243**, 291–303.

Fitzgerald, T.P., Forward, Jr, R.B. & Tankersley, R.A. (1998) Metamorphosis of the estuarine crab *Rhithropanopeus harrisii*: effect of water type and adult odor. *Marine Ecology Progress Series*, **165**, 217–223.

Fleischer, J., Grell, M., Høeg, J.T. & Olesen, J. (1992) Morphology of grooming limbs in species of *Petrolisthes* and *Pachycheles* (Crustacea: Decapoda: Anomura: Porcellanidae): a scanning electron microscopy study. *Marine Biology*, **113**, 425–435.

Fletcher, D., Kotter, I., Wunsch, M. & Yasir, I. (1995) Preliminary observations on the reproductive biology of ornamental cleaner prawns. *International Zoo Yearbook*, **34**, 73–77.

Floeter, S.R., Vasquez, D.P. & Grutter, A.S. (2007) The macroecology of marine cleaning mutualisms. *Journal of Animal Ecology*, **76**, 105–111.

Flüchter, J. (1964) Eine besonders wirksame Aquarienfilterung und die Messung ihrer Leistung. *Helgoland Marine Research*, **11**, 168–170.

Forward, R.B., Jr (1977) Occurrence of a shadow response among brachyuran larvae. *Marine Biology*, **39**, 331–341.

Forward, R.B., Jr (1986) A reconsideration of the shadow response of a larval crustacean. *Marine and Freshwater Behaviour and Physiology*, **12**, 99–113.

Forward, R.B., Jr & Rittschof, D. (2000) Alteration of photoresponses involved in diel vertical migration of a crab larva by fish mucus and degradation products of mucopolysaccharides. *Journal of Experimental Marine Biology and Ecology*, **245**, 277–292.

Forward, R.B., Jr., Frankel, D.A.Z. & Rittschof, D. (1994) Molting to megalopae from the blue crab *Callinectes sapidus*: effects of offshore and estuarine cues. *Marine Ecology Progress Series*, **113**, 55–59.

Forward, R.B., Jr, De Vries, M.C., Rittschof, D., Frankel, D.A.Z., Bischoff, J.P., Fisher, C.M. & Welch, J.M. (1996) Effects of environmental cues on metamorphosis of the blue crab *Callinectes sapidus*. *Marine Ecology Progress Series*, **131**, 165–177.

Forward, R.B., Jr, Tankersley, R.A. & Rittschof, D. (2001) Cues for metamorphosis of brachyuran crabs: an overview. *American Zoologist*, **41**, 1108–1122.

Fosså, S.A. & Nielsen, A.J. (2000) *The Modern Coral Reef Aquarium*, Vol. 3. Birgit Schmettkamp Verlag, Bornheim, Germany.

Fotheringham, N. (1980) Effects of shell utilization on reproductive patterns in tropical hermit crabs. *Marine Biology*, **55**, 287–293.

Froglia, C. & Atkinson, R. (1998) Association between *Athanas amazone* (Decapoda: Alpheidae) and *Squilla mantis* (Stomatopoda: Squillidae). *Journal of Crustacean Biology*, **18**, 529–532.

Fujita, Y. & Osawa, M. (2003) Zoeal development of two spot-pattern morphs of *Neopetrolisthes maculatus*, and of *N. spinatus* (Crustacea: Decapoda: Anomura: Porcellanidae), reared under laboratory conditions. *Species Diversity*, **8**, 175–198.

Fukusho, K. (1980) Mass production of a copepod, *Tigriopus japonicus* in combination culture with a rotifer *Brachionus plicatilis*, fed omega-yeast as a food source. *Bulletin of the Japanese Society of Scientific Fisheries*, **46**, 625–629.

Fukusho, K. (1989) Biology and mass production of the rotifer, *Brachionus plicatilis*. *International Journal of Aquaculture and Fishery Technology*, **1**, 232–240.

Fuller, R. (1989) Probiotics in man and animals. *Journal of Applied Bacteriology*, **66**, 365–378.

Fuller, R. (1992) *Probiotics: the Scientific Basis.* Chapman & Hall, New York, USA.

Gadient, M. & Schai, E. (1994) Leaching of various vitamins from shrimp feed. *Aquaculture*, **124**, 201–205.

Gaffney, P.M., Rubin, V.P., Hedgecock, D., Poweres, D.A., Morris, G. & Hereford, L. (1996) Genetic effects of artificial propagation: signals from wild and hatchery populations of red abalone in California. *Aquaculture*, **143**, 257–266.

Gallagher, M.L. & Brown, W.D. (1976) Comparison of artificial and natural sea water as culture media for juvenile lobsters. *Aquaculture*, **9**, 87–90.

Gallardo-Escárate, C., Goldstein-Vasquez, J. & Thiel, M. (2007) Individual identification of decapod crustaceans I: color patterns in rock shrimp (*Rhynchocinetes typus*). *Journal of Crustacean Biology*, **27**, 393–398.

Gardner, C., Frusher, S., Mills, D. & Oliver, M. (2006) Simultaneous enhancement of rock lobster fisheries and provision of puerulus for aquaculture. *Fisheries Research*, **80**, 122–128.

Gatesoupe, F.J. (1999) The use of probiotics in aquaculture: review. *Aquaculture*, **180**, 147–165.

Gebauer, P., Walter, I. & Anger, K. (1998) Effects of substratum and conspecific adults on the metamorphosis of *Chasmagnathus granulata* (Dana) (Decapoda: Grapsidae) megalopae. *Journal of Experimental Marine Biology and Ecology*, **223**, 185–198.

Gebauer, P., Paschke, K. & Anger, K. (1999) Costs of delayed metamorphosis: reduced growth and survival of early juveniles of an estuarine grapsid crab, *Chasmagnathus granulata*. *Journal of Experimental Marine Biology and Ecology*, **238**, 271–281.

Gebauer, P., Paschke, K. & Anger, K. (2002) Metamorphosis in a semiterrestrial crab, *Sesarma curacaoense*: intra- and interspecific settlement cues from adult odors. *Journal of Experimental Marine Biology and Ecology*, **268**, 1–12.

Gebauer, P., Paschke, K. & Anger, K. (2003) Delayed metamorphosis in decapod crustaceans: evidence and consequences. *Revista Chilena de Historia Natural*, **76**, 169–175.

Gendron, L., Fradette, P. & Godbout, G. (2001) The importance of rock crab (*Cancer irroratus*) for growth, condition and ovary development of adult American lobster (*Homarus americanus*). *Journal of Experimental Marine Biology and Ecology*, **262**, 221–241.

Genodepa, J., Southgate, P. & Zeng, C. (2004a) Diet particle size preference and optimal ration for mud crab, *Scylla serrata*, larvae fed microbound diets. *Aquaculture*, **230**, 493–505.

Genodepa, J., Zeng, C. & Southgate, P. (2004b) Preliminary assessment of a microbound diet as an *Artemia* replacement for mud crab, *Scylla serrata*, megalopa. *Aquaculture*, **236**, 497–509.

George, R.W. (2005) Evolution of life cycles, including migration, in spiny lobsters (Palinuridae). *New Zealand Journal of Marine and Freshwater Research*, **39**, 503–514.

Gerrard, J.A. (2002) Protein–protein crosslinking in food: methods, consequences, applications. *Trends in Food Science and Technology*, **13**, 389–397.

Gherardi, F. & Benvenuto, C. (2001) Clustering behaviour in a Mediterranean population of the hermit crab *Clibanarius erythropus*. *Ophelia*, **55**, 1–10.

Gilchrist, B.M. (1985) Ecology of juvenile hermit crab shell use: field and laboratory comparisons. *American Zoologist*, **25**, 60.

Gilchrist, B.M. & Lee, W.L. (1972) Carotenoid pigments and their possible role in reproduction in the sand crab *Emerita analoga* (Stimpson, 1857). *Comparative Biochemistry and Physiology, Part B*, **42**, 263–294.

Gillikin, D.P., De Grave, S. & Tack, J.F. (2001) The occurrence of the semi-terrestrial shrimp *Merguia oligodon* (De Man, 1888) in *Neosarmatium smithi* H. Milne Edwards, 1853 burrows in Kenyan mangroves. *Crustaceana*, **74**, 505–508.

Gimenez, L. & Anger, K. (2001) Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. *Journal of Experimental Marine Biology and Ecology*, **260**, 241–257.

Gimenez, L. & Anger, K. (2005) Effects of temporary food limitation on survival and development of brachyuran crab larvae. *Journal of Plankton Research*, **27**, 485–494.

Glass, H. & Stark, J. (1995) Carbohydrate digestion in the European lobster *Homarus gammarus* (L.). *Journal of Crustacean Biology*, **15**, 424–433.

Glencross, B.D. & Smith, D.M. (1999) The dietary linoleic and linolenic fatty acids requirements of the prawn *Penaeus monodon*. *Aquaculture Nutrition*, **7**, 101–112.

Glencross, B.D. & Smith, D.M. (2001) Optimizing the essential fatty acids, eicosapentaenoic and docosahexaenoic acid, in the diet of the prawn, *Penaeus monodon*. *Aquaculture Nutrition*, **7**, 101–112.

Glencross, B.D., Smith, D.M., Thomas, M.R. & Williams, K.C. (2002) The effects of dietary lipid amount and fatty-acid composition on the digestibility of lipids by the prawn, *Penaeus monodon*. *Aquaculture*, **205**, 157–169.

Glencross, B.D., Booth, M. & Allan, G.L. (2007) A feed is only as good as its ingredients — a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition*, **13**, 17–34.

Glynn, P.W. (1982) Crustacean symbionts and the defense of corals: coevolution on the reef? In: *Coevolution* (ed. M.H. Nitecki) pp. 111–178. University of Chicago Press, Chicago, Illinois, USA.

Godin, D.M., Carr, W.H., Hagino, G., Segura, F., Sweeney, J.N. & Blankenship, L. (1995) Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. *Aquaculture*, **139**, 243–248.

Gonor, S.L. & Gonor, J.J. (1973) Feeding, cleaning and swimming in larval stages of porcellanid crabs (Crustacea: Anomura). *Fishery Bulletin, U.S.*, **71**, 225–234.

González-Gordillo, J.I., Dos Santos, A. & Rodríguez, A. (2001) Checklist and annotated bibliography of decapod crustacean larvae from the Southwestern European coast (Gibraltar Strait area). *Scientia Marina*, **65**, 275–305.

Gore, R.H. (1985) Molting and growth in decapod larvae. In: *Crustacean Issues*, Vol. 2 (Larval Growth) (ed. A.M. Wenner) pp. 1–65. A.A. Balkema, Rotterdam, The Netherlands.

Gore, R.H., Van Dover, C. & Factor, J. (1981) Studies on decapod Crustacea from the Indian River region of Florida. XVIII. Rediscovery *of Periclimenes (Periclimenes) pandionis* Holthuis, 1951 (Caridea, Palaemonidae) with notes on the males and zoeal stages. *Crustaceana*, **40**, 253–265.

Gotelli, N.J., Gilchrist, S.L. & Abele, L.G. (1985) Population biology of *Trapezia* spp. and other coral-associated decapods. *Marine Ecology Progress Series*, **21**, 89–98.

Govind, C.K. (1992) Nervous system. In: *Microscopic Anatomy of Invertebrates*, Vol. 10 (Decapod Crustacea) (eds F.W. Harrison & A.G. Humes) pp. 395–438. Wiley-Liss, New York, USA.

Goy, J. (1990) Components of reproductive effort and delay of larval metamorphosis in tropical marine shrimp (Crustacea: Decapoda: Caridea and Stenopodidea). PhD thesis, Texas A&M University, College Station.

Greve, W. (1968) The 'planktonkreisel', a new device for culturing zooplankton. *Marine Biology*, **1**, 201–203.

Grguric, G., Trefry, J.H. & Keaffaber, J.J. (1994) Ozonation products of bromine and chlorine in seawater aquaria. *Water Research*, **28**, 1087–1094.

Grimsen, S., Jaques, R.N., Erenst, V. & Balchen, J.G. (1987) Aspects of automation in a lobster farming plant. *Modelling, Identification and Control*, **8**, 61–68.

Grünbaum, D. & Strathmann, R.R. (2003) Form, performance and trade-offs in swimming and stability of armed larvae. *Journal of Marine Research*, **61**, 659–691.

Grutter, A.S. (1994) Spatio-temporal variations of the ectoparasites of seven reef fish species from Lizard Island Heron Island. *Marine Ecology Progress Series*, **115**, 21–30.

Grutter, A.S. (2004) Cleaner fish use tactile dancing behavior as a preconflict management strategy. *Current Biology*, **14**, 1080–1083.

Grutter, A.S., Deveney, M.R., Whittington, I.D. & Lester, R.J.G. (2002) The effect of the cleaner fish *Labroides dimidiatus* on the capsalid monogenean *Benedenia lolo* parasite of the labrid fish *Hemigymnus melapterus*. *Journal of Fish Biology*, **61**, 1098–1108.

Guo, C.-C., Hwang, J.-S. & Fauntin, D.G. (1996) Host selection by shrimps symbiotic with sea anemones: a field survey and experimental laboratory analysis. *Journal of Experimental Marine Biology and Ecology*, **202**, 165–176.

Gupta, A., Sehgal, H.S. & Sehgal, G.K. (2007) Growth and carcass composition of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man), fed different isonitrogenous and isocaloric diets. *Aquaculture Research*, **38**, 1355–1363.

Gurney, R. (1936) Larvae of decapod crustacea. Part I. Stenopidae. Part II. Amphionidae. Part III. Phyllosoma. *Discovery Reports*, **12**, 377–440.

Gurney, R. (1937) Larvae of decapod crustacea. Part IV. Hippolytidae. *Discovery Reports*, **14**, 351–404.

Gurney, R. (1938a) The larvae of the decapod Crustacea Palaemonidae and Alpheidae. *Scientific Reports of the Great Barrier Reef Expedition 1928–29*, **6**, 1–60.

Gurney, R. (1938b) Larvae of decapod crustacea. Part V. Nephropsidea and Thalassinidae. *Discovery Reports*, **17**, 291–344.

Gurney, R. (1942) *Larvae of Decapod Crustacea*. The Ray Society of London, London, UK.

Gurney, R. & Lebour, M.V. (1941) On the larvae of certain Crustacea Macrura, mainly from Bermuda. *Journal of the Linnean Society of London (Zoology)*, **41**, 89–181.

Hacker, S.D. & Madin, L.P. (1991) Why habitat architecture and color are important to shrimp living in pelagic *Sargassum*: use of camouflage and plant-part mimicry. *Marine Ecology Progress Series*, **70**, 143–155.

Hair, C., Warren, R., Tewaki, A., Haro, C. & Phillips, W. (2004) Catching and rearing postlarval cleaner shrimp for the aquarium trade: results from a WorldFish Center project in Solomon Islands. *NAGA, WorldFish Center Quarterly*, **27**, 42–48.

Halim, A. (2002) Adoption of cyanide fishing practice in Indonesia. *Ocean & Coastal Management*, **45**, 313–323.

Hamasaki, K., Suprayudi, M.A. & Takeuchi, T. (2006) Mass mortality during metamorphosis to megalops in the seed production of mud crab *Scylla serrata* (Crustacea, Decapoda, Portunidae). *Fisheries Science*, **68**, 1226–1232.

Handlinger, J.H., Carson, J., Ritar, A.J., Crear, B.J., Taylor, D.P. & Johnson, D.J. (1999) Disease conditions of cultured phyllosoma larvae and juveniles of the southern rock lobster (*Jasus edwardsii*, Decapoda; Palinuridae). In: *Proceedings of the International Symposium on Lobster Health Management* (eds L.H. Evans & J.B. Jones) pp. 75–87. Curtin University of Technology, Adelaide, Australia.

Hari, B. & Kurup, B. M. (2003) Comparative evaluation of dietary protein levels and plant–animal protein ratios in *Macrobrachium rosenbergii* (de Man). *Aquaculture Nutrition*, **9**, 131–137.

Harms, J. (1992) Larval development and delayed metamorphosis in the hermit crab *Clibanarius erythropus* (Latreille) (Crustacea, Diogenidae). *Journal of Experimental Marine Biology and Ecology*, **156**, 151–160.

Harms, J. & Seeger, B. (1989) Larval development and survival in seven decapods species (Crustacea) in relation to laboratory diet. *Journal of Experimental Marine Biology and Ecology*, **133**, 129–139.

Harms, J., Anger, K., Klaus, S. & Seeger, B. (1991) Nutritional effects of ingestion rate, digestive activity, growth and biochemical composition of *Hyas araneus* L. (Decapoda: Majidae). *Journal of Experimental Marine Biology and Ecology*, **145**, 233–265.

Harms, J., Meyer-Harms, B., Dawirs, R.R. & Anger, K. (1994) Growth and physiology of *Carcinus maenas* (Decapoda, Portunidae) larvae in the field and in laboratory experiments. *Marine Ecology Progress Series*, **108**, 107–118.

Harriott, V.J. (2003) Can corals be harvested sustainably? *Ambio*, **32**, 130–133.

Harris, R.P., Samain, J.F., Moal, J., Martin-Jezequel, V. & Poulet, S.A. (1986) Effects of algal diet on digestive activity in *Calanus helgolandicus*. *Marine Biology*, **90**, 353–361.

Harrison, K.E. (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. *Journal of Shellfish Research*, **9**, 1–28.

Hartnoll, R.G. (1982) Growth. In: *The Biology of Crustacea*, Vol. 2 (eds D.E. Bliss & L.G. Abele) pp. 111–196. Academic Press, New York, USA.

Hartnoll, R.G. (1992) Megalopae and early postlarval stages of East African *Percnon*. *Journal of Zoology, London*, **228**, 51–67.

Hartnoll, R.G. & Bryant, A.D. (2001) Growth to maturity of juveniles of the spider crabs *Hyas coarctatus* Leach and *Inachus dorsettensis* (Pennant) (Brachyura: Majidae). *Journal of Experimental Marine Biology and Ecology*, **263**, 143–158.

Harvey, A.W. (1992) Costs and benefits of delayed metamorphosis in the hermit crab *Clibanarius longitarsus*. *American Zoologist*, **32**, 114A.

Harvey, A.W. (1996) Delayed metamorphosis in Florida hermit crabs: multiple cues constraints (Crustacea: Decapoda: Paguridae and Diogenidae). *Marine Ecology Progress Series*, **141**, 27–36.

Harvey, E.A. & Epifanio, C.E. (1997) Prey selection by larvae of the common mud crab *Panopeus herbstii* Milne-Edwards. *Journal of Experimental Marine Biology and Ecology*, **217**, 79–91.

Harvey, M. & Morrier, G. (2003) Laboratory feeding experiments on zoea of northern shrimp *Pandalus borealis* fed with natural zooplankton. *Marine Ecology Progress Series*, **265**, 165–174.

Hazlett, B.A. (1968) Dislodging behavior in European pagurids. *Pubblicazione de la Stazione Zoologica di Napoli*, **36**, 138–139.

Hazlett, B.A. (1971) Influence of rearing conditions on initial shell entering behavior of a hermit crab (Decapoda, Paguridae). *Crustaceana*, **20**, 168–170.

Hazlett, B.A. (1972) Shell fighting and sexual behavior in the hermit crab genera *Paguristes* and *Calcinus*, with comments on *Pagurus*. *Bulletin of Marine Science*, **22**, 806–823.

Hazlett, B.A. (1987) Hermit crab shell exchange as a model system. *Bulletin of Marine Science*, **41**, 99–107.

Hazlett, B.A. & Baron, L.C. (1989) Influence of shells on mating behavior in the hermit crab *Calcinus tibicen*. *Behavioral Ecology and Sociobiology*, **24**, 369–376.

Helland, S., Terjesen, B.F. & Berg, L. (2003) Free amino acid and protein content in the planktonic copepod *Temora longicornis* compared to *Artemia franciscana*. *Aquaculture*, **215**, 213–228.

Hernández-Herrera, R. (2001) *Indicadores bioquímico-fisiológicos de calidad larvaria y postlarvaria de camarón blanco* Litopenaeus vannamei. MS thesis, Centro de Investigaciones Biológicas del Noroeste, México.

Hernández-Herrera, R., Perez-Rostro, C.I., Arcos, F., Ramírez, J.L., Ibarra, A.M., Palacios, E. & Racotta, I.S. (2001) Predictive criteria of shrimp larval quality: an experimental approach. *European Aquaculture Society, Special Publication*, **30**, 242–245.

Hewitt, C.L. & Campbell, M.L. (2007) Mechanisms for the prevention of marine bioinvasions for better biosecurity. *Marine Pollution Bulletin*, **55**, 395–401.

Highsmith, R.C. (1982) Reproduction by fragmentation in corals. *Marine Ecology Progress Series*, **7**, 207–226.

Hinz, S., Sulkin, S.D., Strom, S. & Testermann, J. (2001) Discrimination in ingestion of protistan prey by larval crabs. *Marine Ecology Progress Series*, **222**, 155–162.

Hixon, M.A. (1991) Predation as a process structuring coral-reef fish communities. In: *The Ecology of Fishes on Coral Reefs* (ed. P.F. Sale) pp. 475–508. Academic Press, San Diego, California, USA.

Höglund, H. (1943) On the biology and larval development of *Leander squilla* (L.) forma *typical* De Man. *Svenska Hydrografisk Biologiska Kommissionens Skrifter*, **2**, 1–44.

Hopkins, S., Ako, H. & Tamaru, C.S. (2005) Manual for the production of the Banggai cardinalfish, *Pterapogon kauderni*, in Hawaii (available online at http://www.soest.hawaii.edu/seagrant/communication/pdf/Banggai%20Cardinalfish-Final.pdf).

Houser, R. & Akiyama, D. (1997) Feed formulation principles. In: *Advances in World Aquaculture*, Vol. 6 (Crustacean Nutrition) (eds L. D'Abramo, D. Conklin & D. Akiyama) pp. 493–520. The World Aquaculture Society, Baton Rouge, Louisiana, USA.

Huber, M.E. (1983) Ethology and population biology of *Trapezia*, a xanthid crab symbiotic with reef corals, with special reference to territoriality and speciation. PhD thesis, Scripps Institute of Oceanography, University of California, San Diego.

Huber, M.E (1987) Aggressive behavior of *Trapezia intermedia* Miers and *T. digitalis* Latreille (Brachyura: Xanthidae). *Journal of Crustacean Biology*, **7**, 238–248.

Huber, M.E. & Coles, S.L. (1986) Resources utilization and competition among the five Hawaiian species of *Trapezia* (Crustacea, Brachyura). *Marine Ecology Progress Series*, **30**, 21–31.

Hughes, J.T., Shleser, R.A. & Tchobanoglous, G. (1974) A rearing tank for lobster larvae and other aquatic species. *The Progressive Fish-Culturist*, **36**, 129–132.

Huguenin, J.E. & Colt, J. (1989) *Design and Operating Guide for Aquaculture Seawater Systems*. Elsevier, Amsterdam, The Netherlands.

Hunter, D.A. & Uglow, R.F. (1993) Handling-induced changes in haemolymph ammonia concentration and ammonia excretion rate of *Crangon crangon* (L.). *Ophelia*, **38**, 137–147.

Hyatt, G.W. (1983) Qualitative and quantitative dimensions of crustacean aggression. In: *Studies in Adaption — The Behavior of Higher Crustacea* (eds S. Rebach & D.W. Dunham) pp. 113–139. John Wiley & Sons, New York, USA.

IATA (2007) *Live Animals Regulations Manual*. 34th edn. International Air Transport Association.

Ibarra, A.M., Ramírez, J.L. & Perez-Rostro, C.I. (1998) Effect of positive phototropism selection in nauplii of white shrimp, *Penaeus vannamei*, on growth, survival, and family variance of later developmental stages. *Aquaculture 98 Book of Abstracts, Las Vegas, Nevada, USA*, 261.

Ibarra, A.M., Racotta, I.S., Arcos, F.G. & Palácios, E. (2007) Progress on the genetics of reproductive performance in penaeid shrimp. *Aquaculture*, **268**, 23–43.

Illingworth, J., Tong, L., Moss, G. & Pickering, T. (1997) Upwelling tank for culturing rock lobster (*Jasus edwardsii*) phyllosomas. *Marine and Freshwater Research*, **48**, 911–914.

Ingle, R. & Clark, P.F. (1977) A laboratory module for rearing crab larvae. *Crustaceana*, **32**, 220–222.

Irianto, A. & Austin, B. (2002) Probiotics in aquaculture. *Journal of Fish Disease*, **25**, 633–642.

Jee, J.-H., Koo, J.-G., Keum, Y.-H., Kang, J.-S., Park, K.H.A. & Kang, J.C.(2007) Effects of diet and sediment type on survival, growth and moulting of juvenile tiger crab, *Orithyia sinica* L.. *Aquaculture Research*, **38**, 26–35.

Jeffrey, S.W. & Garland, C.D. (1987) Mass culture of microalgae essential for mariculture hatcheries. *Australian Fisheries*, **46**, 14–18.

Jeffs, A. (2007) Revealing the natural diet of the phyllosoma larvae of spiny lobster. *Bulletin of Fisheries Research Agency*, **20**, 9–13.

Jeffs, A., Nichols, P.D. & Bruce, M.P. (2001) Lipid reserves used by pueruli of the spiny lobster *Jasus edwardsii* in crossing the continental shelf of New Zealand. *Comparative Biochemistry and Physiology, Part A*, **129,** 305–311.

Jeffs, A., Nichols, P.D., Mooney, B.D., Phillips, K.L. & Phleger, C.F. (2004) Identifying potential prey of the pelagic larvae of the spiny lobster *Jasus edwardsii* using signature lipids. *Comparative Biochemistry and Physiology, Part B*, **137**, 487–507.

Jensen, G.C. (1989) Gregarious settlement by megalopae of the crabs *Petrolisthes cinctipes* (Randall) and *P. eriomerus* Stimpson. *Journal of Experimental Marine Biology and Ecology*, **131**, 223–231.

Jensen, G.C. (1991) Competency, settling behavior, and postsettlement aggregation by porcelain crab megalopae (Anomura: Porcellanidae). *Journal of Experimental Marine Biology and Ecology*, **153**, 49–61.

Jernakoff, P. (1990) Distribution of newly settled western rock lobsters *Panulirus cygnus*. *Marine Ecology Progress Series*, **66**, 63–74.

Jha, A. & Homechaudhuri, S. (2001) Understanding the gastric mill structure of a macrophagous shrimp *Metapenaeus monoceros* (Fabricius). *Current Science*, **80**, 620–622.

Jivoff, P. (1997) Sexual competition among male blue crab, *Callinectes sapidus*. *Biological Bulletin*, **193**, 368–380.

Johnsen, S. (2001) Hidden in plain sight: the ecology and physiology of organismal transparency. *Biological Bulletin*, **201**, 301–318.

Johnson, M.L., Gaten, E. & Shelton, P.M.J. (2002) Spectral sensitivities of five marine decapod crustaceans and a review of spectral sensitivity variation in relation to habitat. *Journal of the Marine Biological Association of the United Kingdom*, **82**, 835–842.

Johnson, M. W. (1971) On palinurid and scyllarid lobster larvae and their distribution in the South China Sea (Decapoda, Palinuridae). *Crustaceana*, **21**, 247–282.

Johnson, M.W. & Robertson, P.B. (1970) On the phyllosoma larvae of the genus *Justitia* (Decapoda, Palinuridae). *Crustaceana*, **18**, 283–292.

Johnson, V.R., Jr (1966) Pair formation in the banded shrimp *Stenopus hispidus*. *American Zoologist*, **6**, 534–535.

Johnson, V.R., Jr (1969) Behavior associated with pair formation in the banded shrimp *Stenopus hispidus* (Olivier). *Pacific Science*, **23**, 40–50.

Johnson, V.R., Jr (1977) Individual recognition in the banded shrimp *Stenopus hispidus* (Olivier). *Animal Behaviour*, **25**, 418–428.

Johnston, D.J. & Ritar, A. (2001) Mouthpart and foregut ontogeny in phyllosoma larvae of the spiny lobster *Jasus edwardsii* (Decapoda: Palinuridae). *Marine and Freshwater Research*, **52**, 1375–1386.

Johnston, D.J., Calvert, K.A., Crear, B.J. & Carter, C.G. (2003) Dietary carbohydrate/lipid ratios and nutritional condition in juvenile southern rock lobster, *Jasus edwardsii*. *Aquaculture*, **220**, 667–682.

Johnston, M.D. & Johnston, D.J. (2007) Stability of formulated diets and feeding response of stage I western spiny lobster, *Panulirus cygnus*, phyllosomata. *Journal of the World Aquaculture Society*, **38**, 262–271.

Johnston, M.D., Johnston, D.J., Knott, B. & Jones, C. (2005) Mouthpart and foregut ontogeny in phyllosomata of *Panulirus ornatus* and their implications for development of a formulated larval diet. *European Aquaculture Society, Special Publication*, **36**, 223–226.

Johnston, M.D., Johnston, D.J. & Jones, C.M. (2008) Evaluation of partial replacement of live and fresh feeds with a formulated diet and the influence of weaning *Panulirus ornatus* phyllosomata onto a formulated diet during early ontogeny. *Aquaculture International*, **16**, 33–47.

Jonasson, M. (1987) Fish cleaning behaviour of shrimp. *Journal of Zoology, London*, **213**, 117–131.

Jones, D.A., Kanazawa, A. & Rahman, S.A. (1979) Studies on the presentation of artificial diets for rearing the larvae of *Penaeus japonicus* Bate. *Aquaculture*, **17**, 33–43.

Jones, D.A., Kurmaly, K. & Ashard, A. (1987) Penaeid shrimp hatchery trials using microencapsulated diets. *Aquaculture*, **64**, 133–146.

Jones, D.A, Kamarudin, M.S. & LeVay, L. (1993) The potential for replacement of live feeds in larval culture. *Journal of the World Aquaculture Society*, **24**, 199–210.

Jones, D.A., Kumlu, M. & Fletcher, D.J. (1997a). The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: a review. *Aquaculture*, **155**, 285–295.

Jones, D.A., Yule, A.B. & Holland, D. (1997b) Larval nutrition. In: *Advances in World Aquaculture*, Vol. 6 (Crustacean Nutrition) (eds L.R. D'Abramo & D.E. Conklin) pp. 353–389. World Aquaculture Society, Louisiana State University, Baton Rouge, Louisiana, USA.

Jones, R., Kildea, T. & Hoegh-Guldberg, O. (1999) PAM chlorophyll fluorometry: a new in situ technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing. *Marine Pollution Bulletin*, **38**, 864–874.

Jørstad, K.E., Prodöhl, P.A., Kristiansen, T.S., Hughes, M., Farestveit, E., Taggart, J.B., Agnalt, A.-L. & Ferguson, A. (2005) Communal larval rearing of European lobster (*Homarus gammarus*): family identification by microsatellite DNA profiling and offspring fitness comparisons. *Aquaculture*, **247**, 275–285.

Jousson, O., Pawlowski, J., Zainetti, L., Meinesz, A. & Boudouresques, C.F. (1998) Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. *Marine Ecology Progress Series*, **172**, 275–280.

Jussila, J. & Evans, L. (1998) Growth and condition of marron *Cherax tenuimanus* fed pelleted diets of different stability. *Aquaculture Nutrition*, **4**, 143–149.

Kaestner, A. (1980) *Invertebrate Zoology, Vol. 3 Crustacea*. Krieger, Huntington, New York, USA.

Kamarudin, M.S., Jones, D.A., Vay, L. & Abidin, A.Z. (1994) Ontogenetic changes in digestive enzyme activity during larval development of *Macrobrachium rosenbergii*. *Aquaculture*, **123**, 323–333.

Kanazawa, A. (1989) Protein requirements of penaeid shrimp. *Advances in Tropical Aquaculture, Tahiti, February 20 to March 4, 1989. AQUACOP, IFREMER, France Actes de Colloque*, **9**, 261–270.

Kanazawa, A. & Koshio, S. (1994) Lipid nutrition of the spiny lobster *Panulirus japonicus* (Decapoda, Palinuridae): a review. *Crustaceana*, **67**, 227–232.

Kanazawa, A. & Teshima, S.-I. (1971) In vivo conversion of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonicus*. *Bulletin of the Japanese Society of Scientific Fisheries*, **37**, 891–897.

Kanazawa, A., Teshima, S.-I. & Tokiwa, S. (1979a) Biosynthesis of fatty acids from palmitic acid in the prawn, *Penaeus japonicus*. *Memoirs of the Faculty of Fisheries, Kagoshima University*, **28**, 17–20.

Kanazawa, A., Teshima, S.-I., Ono, K. & Chlayondeja, K. (1979b) Biosynthesis of fatty acids from acetate in the prawns, *Penaeus monodon* and *Penaeus merguiensis*. *Memoirs of the Faculty of Fisheries, Kagoshima University*, **28**, 21–26.

Kanazawa, A., Chim, L. & Laubier, L. (1988) Tissue uptake of radioactive cholesterol in the prawn *Penaeus japonicus* Bate during ovarian maturation. *Aquatic Living Resources*, **1**, 85–91.

Karplus, I. (1987) The association between gobiid fishes and burrowing alpheid shrimps. *Oceanography and Marine Biology Annual Review*, **25**, 507–562.

Karplus, I. (1992) Obligatory and facultative goby–shrimp partnerships in the western tropical Atlantic. *Symbiosis*, **12**, 275–291.

Karplus, I., Szlep, R. & Tsurnamal, M. (1972a) Associative behaviour of the fish *Cryptocentrus cryptocentrus* (Gobiidae) and the pistol shrimp *Alpheus djiboutensis* (Alpheidae) in artificial burrows. *Marine Biology*, **15**, 95–104.

Karplus, I., Tsurnamal, M. & Szlep, R. (1972b) Analysis of the mutual attraction in the association of the fish *Cryptocentrus cryptocentrus* (Gobiidae) and the shrimp *Alpheus djiboutensis* (Alpheidae). *Marine Biology*, **17**, 275–283.

Karplus, I., Szlep, R. & Tsurnamal, M. (1974) The burrows of alpheid shrimp associated with gobiid fish in northern Red Sea. *Marine Biology*, **24**, 259–268.

Karplus, I., Fiedler, G.C. & Ramcharan, P. (1998) The intraspecific fighting behavior of the Hawaiian boxer crab, *Lybia edmonsoni* — fighting with dangerous weapons? *Symbiosis*, **24**, 287–302.

Karplus, I., Gottdiener, M. & Zion, B. (2003) Guidance of single guppies (*Poecilia reticulata*) to allow sorting by computer vision. *Aquacultural Engineering*, **27**, 177–190.

Karplus, I., Alchanatis, V. & Zion, B. (2005) Guidance of groups of guppies (*Poecilia reticulata*) to allow sorting by computer vision. *Aquacultural Engineering*, **32**, 509–520.

Katakura, Y. (1989) Endocrine and genetic control of sex differentiation in the malacostracan Crustacea. *Invertebrate Reproduction and Development*, **16**, 177–182.

Kendall, R.A., Van Olst, J.C. & Carlberg, J.M. (1982) Effects of chelae immobilization on growth and survivorship for individually and communally raised lobsters, *Homarus americanus*. *Aquaculture*, **29**, 359–372.

Keys, S.J., Crocos, P.J., Burridge, C.Y., Coman, G.J., Davis, G.P. & Preston, N.P. (2004) Comparative growth and survival of inbred and outbred *Penaeus (marsupenaeus) japonicus*, reared under controlled environment conditions: indications of inbreeding depression. *Aquaculture*, **241**, 151–168.

Khachatourians, G.G. (1998) Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association*, **159**, 1129–1136.

Khan, R.N., Becker, J.H.A., Crowther, A.L. & Lawn, I.D. (2003) Sea anemone host selection by the symbiotic saddled cleaner shrimp *Periclimenes holthuisi*. *Marine and Freshwater Research*, **54**, 653–656.

Khan, R.N., Becker, J.H.A., Crowther, A.L. & Lawn, I.D. (2004) Spatial distribution of symbiotic shrimps (*Periclimenes holthuisi*, *P. bervicarpalis*, *Thor amboinensis*) on the sea anemone *Stichodactyla haddoni*. *Journal of the Marine Biological Association of the United Kingdom*, **84**, 201–203.

Kinzie, R.A., Jokiel, P.L. & York, R. (1984) Effects of light of altered spectral composition on coral zooxanthellae associations and on zooxanthellae *in vitro*. *Marine Biology*, **78**, 239–248.

Kishida, M., Anderson, T.R. & Specker, J.L. (1992) Induction by β-estradiol of vitellogenin in striped bass (*Morone saxatilis*): characterization and quantification in plasma and mucus. *General and Comparative Endocrinology*, **88**, 29–39.

Kitabayashi, K., Shuda, K., Kakamura, K. & Ishikaua, S. (1971) Studies on formula feed for Kuruma prawn on the growth-promoting effects of protein level in a diet and re-examination of ingredients used. *Bulletin of Tokai Regional Fisheries Research Laboratory*, **65**, 139–147.

Kittaka, J. (1994a) Larval rearing. In: *Spiny Lobsters Management* (eds B.F. Phillips, J.S. Cobb & J. Kittaka) pp. 402–423. Fishing News Books, Blackwell Scientific Publications, Oxford, UK.

Kittaka, J. (1994b) Application of ecosystem culture method for complete development of phyllosomas of spiny lobster. *Aquaculture*, **155**, 319–331.

Kittaka, J. (1994c) Culture of phyllosomas of spiny lobster and its application to studies of larval recruitment and aquaculture. *Crustaceana*, **66**, 258–270.

Kittaka, J. (1997) Application of ecosystem method for complete development of phyllosomas of spiny lobster. *Aquaculture*, **155**, 319–331.

Kittaka, J. (1999) Importance of three major factors for successful phyllosoma culture. In: *Proceedings of the 1999 FRDC Rock Lobster Propagation Workshop* (eds P.R. Hart & R. van Barneveld) pp. 37–41. Fisheries Research and Development Corporation, Hobart, Australia.

Kittaka, J. (2000) Culture of larval spiny lobsters. In: *Spiny Lobsters: Fisheries and Culture* 2nd edn (eds B.F. Phillips & J. Kittaka) pp. 508–532. Blackwell Science, Oxford, UK.

Knowlton, N. (1980) Sexual selection and dimorphism in two demes of a symbiotic, pair-bonding snapping shrimp. *Evolution*, **34**, 161–173.

Knowlton, R.E. (1974) Larval developmental processes and controlling factors in decapod Crustacea, with emphasis on the Caridea. *Thalassia Jugoslavica*, **10**, 138–158.

Knudsen, J.W. (1967) *Trapezia* and *Tetralia* (Decapoda, Brachyura, Xanthidae) as obligate ectoparasites of pocilloporid and acroporid reef corals. *Pacific Science*, **21**, 51–57.

Ko, H.S. & Clark, P.F. (2002) The zoeal development of *Nanocassiope granulipes* (Sakai, 1939) (Crustacea: Decapoda: Brachyura: Xanthidae) described from laboratory-reared material. *Journal of Natural History*, **36**, 1463–1488.

Kolm, N. & Berglund, A. (2003) Wild populations of a reef fish suffer from the 'nondestructive'aquarium trade fishery. *Conservation Biology*, **17**, 910–914.

Kovalenko, E., D'Abramo, L., Ohs, C. & Buddington, R. (2002) A successful microbound diet for the larval culture of freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, **210**, 385–395.

Kraul, S. (1999) Commercial culture of the harlequin shrimp *Hymenocera picta* and other ornamental marine shrimp. *Marine Ornamentals 99. Hawaii, USA. Book of Abstracts*, 50.

Kristensen J.H. (1972) Carbohydrases of some marine invertebrates with notes on their food and the natural occurrence of the carbohydrates studied. *Marine Biology*, **14**, 130–142.

Kristiansen, T.S., Drengstig, A., Bergheim, A., Drengstig, T., Svensen, R., Kollsgård, I., Nøstvold, E., Farestveit, E. & Aardal, L. (2004) Development of methods for intensive farming of European lobster in recirculated seawater. Results from experiments conducted at Kvitsøy lobster hatchery from 2000 to 2004. *Fisken og havet, No 6-2004.*

Krumins, V., Ebeling, J. & Wheaton, F. (2001) Part-day ozonation for nitrogen and organic carbon control in recirculating aquaculture systems. *Aquacultural Engineering*, **24**, 231–241.

Kruschwitz, L.G. (1967) Aspects of the behavior and ecology of a reef shrimp. *American Zoologist*, **7**, 204–205.

Kulbicki, M. & Arnal, C. (1999) Cleaning of fish ectoparasites by a Palaemonidae shrimp on soft bottoms in New Caledonia. *Cybium*, **23**, 101–104.

Kulkarni, G.K. & Nagabhushanam, R. (1979) Mobilization of organic reserves during ovarian development in a marine penaeid prawn, *Parapenaeopsis hardwickii* (Miers) (Crustacea, Decapoda, Penaeidae). *Aquaculture*, **18**, 373–377.

Kumlu, M. & Jones, D.A. (1995) Feeding and digestion in the caridean shrimp larva of *Palaemon elegans* Rethke and *Macrobrachium rosenbergii* (De Mann) (Crustacea: Palaemonidae) on live and artificial diets. *AquacultureNutrition*, **1**, 3–12.

Kuris, A.M. (1991) A review of patterns and causes of crustacean brood mortality. In: *Crustacean Issues*, Vol. 7 (Crustacean Egg Production) (eds A. Wenner & A. Kuris) pp. 117–141. A.A. Balkema, Rotterdam, The Netherlands.

Kurmaly, K., Jones, D.A. & Yule, A.B. (1990) Acceptability and digestion of diets fed to larval stages of *Homarus gammarus* and their role of dietary conditioning behaviour. *Marine Biology*, **106**, 181–190.

Lacoste, A., Poulet, S.A., Cueff, A., Kattner, G., Ianora, A. & Laabir, M. (2001) New evidence of the copepod maternal food effects on reproduction. *Journal of Experimental Marine Biology and Ecology*, **259**, 85–107.

Lafontaine, Y. de & Leggett, W.C. (1987) Evaluation of *in situ* enclosures for larval fish studies. *Canadian Journal of Fisheries and Aquatic Science*, **44**, 54–65.

Lárez, M.B., Palazón-Fernandez, J.L. & Bolaños, C.J. (2000) The effect of salinity and temperature on the larval development of *Mithrax caribbeus* Rathbun, 1920 (Brachyura: Majidae) reared in laboratory. *Journal of Plankton Research*, **22**, 1855–1869.

Lassig, B.R. (1977) Communication and coexistence in a coral community. *Marine Biology*, **42**, 85–92.

Laughlin, R.B., Jr & French, W. (1989) Interactions between temperature and salinity during brooding on subsequent zoeal development of the mud crab *Rhithropanopeus harrisii*. *Marine Biology*, **102**, 377–386.

Lautier, J. & Lagarrigue, J.G. (1988). Lipid metabolism of the crab *Pachygrapsus marmoratus* during vitellogenesis. *Biochemical Systematics and Ecology*, **16**, 203–212.

Lavens, P. & Sorgeloos, P. (2000) Experiences on importance of diet for shrimp postlarval quality. *Aquaculture*, **191**, 169–176.

Laverack, M.S. (1988) The diversity of chemoreceptors. In: *Sensory Biology of Aquatic Animals* (ed. J. Atema) pp. 287–317. Springer-Verlag, New York, USA.

Le Vay, L., Jones, D.A., Puello-Cruz, A.C., Sangha, R.S. & Ngamphongsai, C. (2001) Digestion in relation to feeding strategies exhibited by crustacean larvae. *Comparative Biochemistry and Physiology, Part A*, **128**, 623–630.

Leaño, E.M. & Liao, I.C. (2006) Tagging of *Litopenaeus vannamei* (Boone) by uropod cutting: a comparison of two methods. *Aquaculture Research*, **37**, 885–890.

Lebour, M.V. (1922) The food of plankton organisms. *Journal of the Marine Biological Association of the United Kingdom*, **12**, 644–677.

Lebour, M.V. (1925) Young anglers in captivity and some of their enemies. A study in a plunger jar. *Journal of the Marine Biological Association of the United Kingdom*, **13**, 721–734.

Lebour, M.V. (1940) The larvae of the British species of *Spirontocaris* and their relation to *Thor* (Crustacea, Decapoda). *Journal of the Marine Biological Association of the United Kingdom*, **24**, 505–514.

Lebour, M.V. (1943) The larvae of the genus *Porcellana* (Crustacea, Decapoda) and related forms. *Journal of the Marine Biological Association of the United Kingdom*, **25**, 721–737.

Lecaillon, G. (2004) The 'C.A.R.E.' (collect by artificial reef eco-friendly) system as a method of producing farmed marine animals for the aquarium market: an alternative solution to collection in the wild. *Secretariat of the Pacific Community Live Reef Fish Information Bulletin*, **12**, 17–20.

Lecchini, D., Polti, S., Nakamura, Y., Mosconi, P., Tsuchiya, M., Remoissenet, G. & Planes, S. (2006) New perspectives on aquarium fish trade. *Fisheries Science*, **72**, 40–47.

Lee, R.F. & Puppione, D.L. (1978) Serum lipoproteins in the spiny lobster, *Palinurus interruptus*. *Comparative Biochemistry and Physiology*, **59**, 239–243.

Lee, R.F. & Puppione, D.L. (1988) Lipoproteins I and II from the hemolymph of the blue crab *Callinectes sapidus*: lipoprotein II associated with vitellogenesis. *Journal of Experimental Zoology*, **248**, 278–289.

Léger, P., Bengtson, D.A., Simpson, K.L. & Sorgeloos, P. (1986) The use and nutritional value of *Artemia* as a food source. *Marine Biology and Oceanography: An Annual Review*, **24**, 521–623.

Lemmens, J.W.T.J. (1994) Biochemical evidence for absence of feeding in puerulus larvae of the western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae). *Marine Biology*, **118**, 383–391.

Lemmens, J.W.T.J. (1995) Nucleic acid levels, cellular activity and growth during the puerulus stage of the western rock lobster (*Panulirus cygnus* (George); Decapoda: Palinuridae). *Journal of Experimental Marine Biology and Ecology*, **194**, 143–156.

Lemos, D. & Rodriguez, A. (1998) Nutritional effects on body composition, energy content and trypsin activity of *Penaeus japonicus* during early postlarval development. *Aquaculture*, **160**, 103–116.

León, C.J., Hernández, J.M. & León-Santana, M. (2006) The effects of water temperature in aquaculture management. *Applied Economics*, **38**, 2159–2168.

Lester, L.J. (1983) Developing a selective breeding program for penaeid shrimp mariculture. *Aquaculture*, **33**, 41–50.

Li, Z., Li, J., Wang, Q., He, Y. & Liu, P. (2006) The effects of selective breeding on the genetic structure of shrimp *Fenneropenaeus chinensis* populations. *Aquaculture*, **258**, 278–282.

Liltved, H., Hektoen, H. & Efraimsen, H. (1995) Inactivation of bacterial and viral fish pathogens by ozonation or UV irradiation in water of different salinity. *Aquacultural Engineering*, **14**, 107–122.

Lim, C. & Persyn, A. (1989) Practical feeding — penaeid shrimps. In: *Nutrition and Feeding of Fish* (ed. T. Lovell) pp. 205–222. Van Nostrand Reinhold, New York, USA.

Limbaugh, C. (1961) Cleaning symbiosis. *Scientific American*, **205**, 42–49.

Limbaugh, C., Pederson, H. & Chace, F.A. (1961) Shrimps that clean fishes. *Bulletin of Marine Science of the Gulf and Caribbean*, **11**, 237–257.

Lin, J. & Zhang, D. (2001a) Reproduction in a simultaneous hermaphroditic shrimp *Lysmata wurdemanni*: any two will do? *Marine Biology*, **139**, 919–922.

Lin, J. & Zhang, D. (2001b) Effects of broodstock diet on reproductive performance of the peppermint shrimp *Lysmata wurdemanni*. *Journal of Shellfish Research*, **20**, 361–363.

Lin, J. & Shi, P. (2002) Effects of broodstock diets on reproductive performance of the golden banded shrimp, *Stenopus scutellatus*. *Journal of the World Aquaculture Society*, **33**, 383–386.

Lin, J., Zhang, D. & Rhyne, A.L. (2002) Broodstock and larval nutrition of marine ornamental shrimp. In: *Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola*. (eds L.E. Cruz-Suárez, D. Ricque-Marie, M. Tapia-Salazar, M.G. Gaxiola-Cortés & N. Simões) pp. 277–280. Cancún, Quintana Roo, México.

Lin, X. & Omori, M. (1993) Effect of tank colouration on the feeding rates of zoeal larvae of the giant freshwater shrimp *Macrobrachium rosenbergii*. *Bulletin of the Plankton Society of Japan*, **40**, 19–25.

Liñán-Cabello, M.A., Paniagua-Michel, J. & Hopkins, P.M. (2002) Bioactive roles of carotenoids and retinoids in crustaceans. *Aquaculture Nutrition*, **8**, 299–309.

Liñán-Cabello, M.A., Paniagua-Michel, J. & Zenteno-Savín, T. (2003) Carotenoids and retinal levels in captive and wild shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*, **9**, 383–389.

Liñán-Cabello, M.A., Medina-Zendejas, R., Sánchez-Barajas, M. & Herrera, A.M. (2004) Effects of carotenoids and retinol in oocyte maturation of crayfish *Cherax quadrucarinatus*. *Aquaculture Research*, **35**, 905–911.

Lokkeborg, S. (1990) Rate of release of potential feeding attractants from natural and artificial bait. *Fisheries Research*, **8**, 253–261.

López-Alvarado, J., Langdon, C., Teshima, S. & Kanazawa, A. (1994) Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture*, **122**, 335–346.

Lovell, E. (2001) *Status report: collection of coral and other benthic reef organisms for the marine aquarium trade and curio trade in Fiji*. World Wildlife Fund South Pacific Programme, Suva, Fiji.

Lovett, D.L. & Felder, D.L. (1989) Ontogeny of gut morphology in the white shrimp *Penaeus setiferus* (Decapoda, Penaeidae). *Journal of Morphology*, **201**, 253–272.

Lovett, D.L. & Felder, D.L. (1990a) Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). *Biological Bulletin*, **178**, 144–159.

Lovett, D.L. & Felder, D.L. (1990b) Ontogenetic changes in enzyme distribution and midgut function in developmental stages of *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). *Biological Bulletin*, **178**, 160–174.

Lubzens, E., Marko, A. & Tietz, A. (1985) *De novo* synthesis of fatty acids in the rotifer *Brachionus plicatilis*. *Aquaculture*, **47**, 27–37.

Lunn, K.E. & Moreau, M.-A. (2004) Unmonitored trade in marine ornamental fishes: the case of Indonesia's Banggai cardinalfish (*Pterapogon kauderni*). *Coral Reefs*, **23**, 344–351.

Luther, W. (1958) Symbiose von Fischen (Gobiidae) mit einem Krebs (*Alpheus djiboutensis*) in Roten Meer. *Zeitschrift für Tierpsychologie*, **15**, 175–177.

Lytle, J.S., Lytl, T.F. & Ogle, J. (1990) Polyunsaturated fatty acid profiles as a comparative tool in assessing maturation diets of *Penaeus vannamei*. *Aquaculture,* **89**, 287–299.

Mackie, A.M. (1973) The chemical basis of food detection in the lobster *Homarus gammarus*. *Marine Biology*, **21**, 103–108.

Macmillan, D., Sandow, S., Wikeley, D. & Frusher, S. (1997) Feeding activity and the morphology of the digestive tract in stage-I phyllosoma larvae of the rock lobster *Jasus edwardsii*. *Marine and Freshwater Research*, **48**, 19–26.

Magnus, D.B.E. (1967) Zur Ökologie sedimentbewohnender *Alpheus* Garnelen (Decapoda: Natantia) der Roten Meeres. *Helgoländer Wissenschaften Meeresuntersuchen*, **15**, 506–522.

Mahnken, C. (1972) Observations on cleaner shrimps of the genus *Periclimenes*. *Bulletin of the Natural History Museum of Los Angeles County*, **14**, 71–83.

Maihara, Y. & Kyoya, N. (2001) Larval stage of the rhynchocinetid shrimp, *Cinetorhynchus striatus* (Nomura & Hayashi, 1992) (Decapoda, Caridea, Rhynchocinetidae) reared in the laboratory. *Scientific Reports of the Museum Tokai University*, **3**, 43–62.

Malecha, S.R., Nevin, P.A., Ha, P., Barck, L.E., Lamadrid-Rose, Y., Masuno, S. & Hedgecock, D. (1992) Sex-ratios and sex-determination in progeny from crosses of surgically sex-reversed freshwater prawns, *Macrobrachium rosenbergii*. *Aquaculture*, **105**, 201–218.

Manor, R., Segev, R., Leibovitz, M.P., Aflalo, E.D. & Sagi, A. (2002) Intensification of redclaw crayfish *Cherax quadricarinatus* culture. II. Growout in a separate cell system. *Aquacultural Engineering*, **26**, 263–276.

Manor, R., Weil, S., Oren, S., Glazer, L., Aflalo, E.D., Ventura, T., Chalifa-Caspi, V., Lapidot, M. & Sagi, A. (2007) Insulin and gender: an insulin-like gene expressed exclusively in the androgenic gland of the male crayfish. *General and Comparative Endocrinology*, **150**, 326–336.

Mantelatto, F.L.M. & Garcia, R.B. (2000) Reproductive potential of the hermit crab *Calcinus tibicen* (Anomura) from Ubatuba, Sǎo Paulo, Brazil. *Journal of Crustacean Biology*, **19**, 168–275.

Mantelatto, F.L.M., Faria, F.C.R. & Garcia, R.B. (2003) Biological aspects of *Mithraculus forceps* (Brachyura: Mithracidae) from Anchieta Island, Ubatuba, Brazil. *Journal of the Marine Biological Association of the United Kingdom*, **83**, 789–791.

Mantiri, D.M.H., Negre Sadargues, G., Castillo, R. & Trilles, J.P. (1995) Evolution of carotenoid metabolic capabilities during the early development of the European lobster *Homarus gammarus* (Linne, 1758). *Comparative Biochemistry and Physiology, Part B*, **111**, 553–558.

Marcus, N.H. (2005) Calanoid copepods, resting eggs, and aquaculture. In: *Copepods in Aquaculture* (eds C.-S. Lee, P.J. O'Bryen & N.H. Marcus) pp. 3–10. Blackwell Publishing, Ames, Iowa, USA.

Marin, I.N., Anker, A., Britayev, T.A. & Palmer, A.R. (2005) Symbiosis between the alpheid shrimp *Athanas ornithorhynchus* Banner and Banner, 1973 (Crustacea: Decapoda) and the brittle star *Macrophiothrix longipeda* (Lamarck, 1816) (Echinodermata: Ophiuroidea). *Zoological Studies*, **44**, 234–242.

Markham, J.C. (1986) Evolution and zoogeography of the Isopoda Bopyridae, parasites of Crustacea Decapoda. In: *Crustacean Biogeography*, Vol. 4 (Crustacean Issues) (eds R.H. Gore & K.L. Heck) pp. 143–164. A.A. Balkema, Rotterdam, The Netherlands.

Marques, A., Huynh Thanh, T., Sorgeloos, P. & Bossier, P. (2005) Effects of bacteria on *Artemia franciscana* cultured in different gnotobiotic environments. *Applied and Environmental Microbiology*, **71**, 4307–4317.

Marques, A., Huynh Thanh, T., Sorgeloos, P. & Bossier, P. (2006) Use of microalgae and bacteria to enhance protection of gnotobiotic *Artemia* against different pathogens. *Aquaculture*, **258**, 116–126.

Marsden, G.E., McGuren, J.J. & Hansford, S.W. (1997) A moist artificial diet for prawn broodstock: its effect on the variable reproductive performance of wild caught *Penaeus monodon*. *Aquaculture*, **149**, 145–156.

Martin, J.W. & Davis, G.E. (2001) An updated classification of the recent Crustacea. *Science Series Natural History Museum of Los Angeles County*, **39**, 1–124.

Masschelein, W.J. (1998) Ozone generation: use of air, oxygen, or air simpsonized with oxygen. *Ozone Science and Engineering*, **20**, 191–203.

Matsuda, H. & Takenouchi, T. (2005) New tank design for larval culture of Japanese spiny lobster, *Panulirus japonicus*. *New Zealand Journal of Marine and Freshwater Research*, **39**, 279–285.

Matsuda, H., Takenouchi, T. & Yamakawa, T. (2003) Diel timing of molting and metamorphosis of *Panulirus japonicus* phyllosoma larvae under laboratory conditions. *Fisheries Science*, **69**, 124–130.

Mauchline, J. (1998) The biology of calanoid copepods. *Advances in Marine Biology*, **33**, 1–710.

Mayr, E. (1960) The emergence of evolutionary novelties. In: *Evolution after Darwin*, Vol. 1 (The Evolution of Life, Its Origin, History and Future) (ed. S. Tax) pp. 349–380. University of Chicago Press, Chicago, Illinois, USA.

McAllen, R. & Scott, G.W. (2000) Behavioural effects of biofouling in marine copepods. *Journal of the Marine Biological Association of the United Kingdom*, **80**, 369–370.

McConaugha, J.R., Tester, P.A & McConaugha, C.S. (1991) Feeding and growth in meroplanktonic larvae of *Callinectes sapidus* (Crustacea: Portunidae). *Memoirs of the Queensland Museum*, **31**, 320.

McKinnon, A.D., Duggan, S., Nichols, P.D., Rimmer, M.A., Semmens, G. & Robino, B. (2003) The potential of tropical paracalanid copepods as live feeds in aquaculture. *Aquaculture*, **223**, 89–106.

McVeigh, S. (2002) Africans look to lobsters on land. *Fish Farming International*, **29**, 22–23.

McWilliam, P.S. & Phillips, B.F. (1997) Metamorphosis of the final phyllosoma and secondary lecithotrophy in the puerulus of *Panulirus cygnus* George: a review. *Marine and Freshwater Research*, **48**, 783–790.

Mead, J.W. (1985) Allowable ammonia for fish culture. *Progress in Fish Culture*, **47**, 135–147.

Meinesz, A. & Hesse, B. (1991) Introduction et invasion de l'algue tropicale *Caulerpa taxifolia* en Mediterranée nord-occidentale. *Oceanologica Acta*, **14**, 415–426.

Menasveta, P. (2002) Improved shrimp growout systems for disease prevention and environmental sustainability in Asia. *Reviews in Fisheries Science*, **10**, 391–402.

Mengqing, L., Wenjuan, J., Qing, C. & Jialin, W. (2004) The effect of vitamin A supplementation in broodstock feed on reproductive performance and larval quality in *Penaeus chinensis*. *Aquaculture Nutrition*, **10**, 295–300.

Merchie, G., Lavens, P. & Sorgeloos, P. (1997) Optimization of dietary vitamin C in fish and crustacean larvae: a review. *Aquaculture*, **155**, 165–181.

Meusy, J.J. (1980) Vitellogenin, the extraovarian precursor of the protein yolk in Crustacea: a review. *Reproduction, Nutrition, Development*, **20**, 1–21.

Meyers, S.P. (1994) Developments in world aquaculture, feed formulations, and role of carotenoids. *Pure and Applied Chemistry*, **66**, 1069–1076.

Meyer-Harms, B. & Harms, J. (1993) Detection of phytoplankton pigments by HPLC in *Hyas araneus* larvae (Crustacea: Decapoda): comparison of field and laboratory samples. *Netherlands Journal of Sea Research*, **31**, 153–161.

Michel, A. (1971) Note sur les puerulus de Palinuridae et les larves phyllosomes de *Panulirus homarus* (L.). Clef de détermination des larves phyllosomes récoltées dans le Pacifique équatorial et sud-tropical (décapodes). *Cahiers ORSTOM, Série Océanographie*, **9**, 459–473.

Mickelsen, R., Infranger, R. & Heckmann, R. (1978) Culturing the American lobster (*Homarus americanus*) using a vertically stacked cage system. *Proceedings of World Mariculture Society*, **9**, 723–730.

Middleditch, B.S., Missler, S.R., Hines, H.B, McVey, J.B., Brown, A., Ward, D.G. & Lawrence, A.L. (1980) Metabolic profiles of penaeid shrimp: dietary lipids and ovarian maturation. *Journal of Chromatography*, **195**, 359–368.

Mikami, S. & Greenwood, J.G. (1997) Influence of light regimes on phyllosomal growth and timing of moulting in *Thenus orientalis* (Lund) (Decapoda: Scyllaridae). *Marine and Freshwater Research*, **48**, 777–782.

Millamena, O.M., Aujero, E.J. & Borlongan, L.G. (1990) Techniques on algal harvesting and preservation for use in culture and as larval food. *Aquacultural Engineering*, **9**, 295–304.

Millikin, M.R., Biddle, G.N., Siewicki, T.C., Fortner, A.R. & Fair, P.H. (1980) Effects of various levels of dietary protein on survival, molting frequency and growth of juvenile blue crabs (*Callinectes sapidus*). *Aquaculture*, **19**, 149–161.

Mills, D.J. & Crear, B. (2001) Outcomes from commercial southern rock lobster puerulus collection research: industry information paper. Tasmania Aquaculture & Fisheries Institute, University of Tasmania, Taroona, Australia.

Mills, D.J. & Crear, B. (2004) Developing a cost-effective puerulus collector for the southern rock lobster (*Jasus edwardsii*) aquaculture industry. *Aquacultural Engineering*, **31**, 1–15.

Minagawa, M. (1994) Effects of photoperiod on survival, feeding and development of larvae of the red frog crab, *Ranina ranina*. *Aquaculture*, **120**, 105–114.

Mock, C.R., Fontaine, C.T. & Revera, D.B. (1980) Improvements in rearing larval penaeid shrimp by the Galveston Laboratory method. In: *The Brine Shrimp Artemia, Vol. 3* (Ecology, Culturing, Use in Aquaculture) (eds G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers) pp. 331–342. Universa Press, Wetteren, Belgium.

Morais, S., Narciso, L., Calado, R., Nunes, M.L. & Rosa, R. (2002) Lipid dynamics during the embryonic development of *Plesionika martia martia* (Decapoda; Pandalidae), *Palaemon serratus* and *Palaemon elegans* (Decapoda; Palaemonidae): relation to metabolic consumption. *Marine Ecology Progress Series*, **242**, 195–204.

Morgan, S.G. (1989) Adaptive significance of spination in estuarine crab zoeae. *Ecology*, **70**, 464–482.

Morgan, S.G. (1990) Impact of planktivorous fishes on dispersal, hatching, and morphology of estuarine crab larvae. *Ecology*, **71**, 1639–1652.

Morgan, S.G. (1992) Predation by planktonic and benthic invertebrates on larvae of estuarine crabs. *Journal of Experimental Marine Biology and Ecology*, **163**, 91–110.

Morgan, S.G. & Christy, J.H. (1997) Planktivorous fishes as selective agents for reproductive synchrony. *Journal of Experimental Marine Biology and Ecology*, **209**, 89–101.

Moriarty, D.J.W. (1999) Disease control in shrimp aquaculture with probiotic bacteria. In: *Proceedings of the 8th International Symposium on Microbial Ecology* (eds C.R. Bell, M. Brylinsky & P. Johnson-Green) pp. 237–243. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

Moss, D.R., Arce, S.M., Otoshi, C.A., Doyle, R.W. & Moss, S.M. (2007) Effects of inbreeding on survival and growth of Pacific white shrimp *Penaeus (Litopenaeus) vannamei*. *Aquaculture*, **272**, 30–37.

Mourente, G. (1996) In vitro metabolism of C-14-polyunsaturated fatty acids in midgut gland and ovary cells from *Penaeus kerathurus* Forskal at the beginning of sexual maturation. *Comparative Biochemistry and Physiology*, **115**, 255–266.

Muir, P.C., Sutton, C.D. & Owens, L. (1991) Nitrate toxicity to *Penaeus monodon* protozoea. *Marine Biology*, **108**, 67–71.

Naas, K.E., Naes, T. & Harboe, T. (1992) Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus*) in green water. *Aquaculture*, **105**, 143–156.

Naessens, E., Lavens, P., Gomez, L., Browdy, C.L., McGovern-Hopkins, K., Spencer, A.W., Kawahigashi, D. & Sorgeloos, P. (1997) Maturation performance of *Penaeus vannamei* co-fed *Artemia* biomass preparations. *Aquaculture*, **155**, 87–101.

Nagabhushanam, R. & Kulkarni, G.K. (1981) Effect of exogenous testosterone on the androgenic gland and testis of a marine penaeid prawn, *Parapenaeopsis hardwickii* (Miers) (Crustacea, Decapoda, Penaeidae). *Aquaculture*, **23**, 19–27.

Nanton, D.A. & Castell, J.D. (1999) The effects of temperature and dietary fatty acids on the fatty acid composition of harpaticoid copepods for use as live food for marine fish larvae. *Aquaculture*, **175**, 167–181.

Narciso, L. & Morais, S. (2001) Comparative feeding behaviour of *Penaeus kerathurus* (Forskål, 1775) (Crustacea, Penaeidae) and *Palaemon serratus* (Pennant, 1777) (Crustacea, Palaemonidae) during larval development. *Ciencias Marinas*, **27**, 559–575.

Narciso, L., Pousão-Ferreira, P., Passos, A. & Luís, O. (1999) HUFA content and DHA/EPA improvements of *Artemia* sp. with commercial oils during different enrichment periods. *Aquaculture Research*, **30**, 21–24.

Nascimento, I.A., Bray, W.A., Leung-Trujillo, J.R. & Lawrence, A. (1991) Reproduction of ablated and unablated *Penaeus schmitti* in captivity using diets consisting of fresh-frozen natural and dried formulated feeds. *Aquaculture*, **99**, 387–398.

Nelson, M.M., Mooney, B.D., Nichols, P.D. & Phleger, C.F. (2001) Lipid of Antarctic Ocean amphipods: food chain interactions and the occurrence of novel biomarkers. *Marine Chemistry*, **73**, 53–64.

New, M.B. (2005) Freshwater prawn farming: global status, recent research and a glance at the future. *Aquaculture Research*, **36**, 210–230.

Nichols, P.D., Holdsworth, D.G., Volkman, J.K., Daintith, M. & Allanson, S. (1989) High incorporation of essential fatty acids by the rotifer *Brachionus plicatilis* fed on the prymnesiophyte alga *Pavlova lutheri*. *Australian Journal of Marine and Freshwater Research*, **40**, 645–655.

Nicosia, F. & Lavalli, K. (1999) Homarid lobster hatcheries: their history and role in research, management, and aquaculture. *Marine Fisheries Review*, **61**, 1–57.

Nilsen, A.J. & Fosså, S.A. (2002) *Reef Secrets.* Microcosm Ltd., Charlotte and T.F.H. Publications Inc., Neptune City, New Jersey, USA.

Nishida, S. & Kittaka, J. (1992) Integumental organs of the phyllosoma larva of the rock lobster *Jasus edwardsii* (Hutton). *Journal of Plankton Research*, **14**, 563–573.

Nizinski, M.S. (1989) Ecological distribution, demography and behavioral observations on *Periclimenes anthophilus*, an atypical symbiotic cleaner shrimp. *Bulletin of Marine Science*, **45**, 174–188.

Noël, P.Y. (1981) Hormonal control of chromatophores in Crustacea, review and perspectives. *Journal of Scientific and Industrial Research*, **40**, 269–276.

Noël, P.Y. (1983) Observations sur la pigmentation et la physiologie chromatique de *Periclimenes amethysteus* (Crystacea, Caridea). *Canadian Journal of Zoology*, **61**, 153–162.

Nouvel, L. (1939) Observation de l'accouplement chez une espèce de crevette *Crangon crangon*. *Compte Rendu de l'Academie des Sciences de Paris*, **209**, 639–641.

Obrebski, S. (1979) Larval colonizing strategies in marine benthic invertebrates. *Marine Ecology Progress Series*, **1**, 293–300.

O'Brien, J. & Van Wyk, P.M. (1985) Effects of crustacean parasitic castrators (epicaridean isopods and rhizocephalan barnacles) on growth of crustacean hosts. In: *Factors in Adult Growth*, Vol. 3 (Crustacean Issues) (ed. A.M. Wenner) pp. 191–218. A.A. Balkema, Rotterdam, The Netherlands.

O'Connor, N. (1991) Flexibility in timing of metamorphic molt by fiddler crab megalopae *Uca pugilator*. *Marine Ecology Progress Series*, **68**, 243–247.

O'Connor, N. & Judge, M.L. (1997) Flexibility in timing of molting of fiddler crab megalopae: evidence from in situ manipulation of cues. *Marine Ecology Progress Series*, **146**, 55–60.

O'Connor, N. & Gregg, A.S. (1998) Influence of potential habitat cues on duration of megalopal stage of the fiddler crab *Uca pugnax*. *Journal of Crustacean Biology*, **18**, 700–709.

Odinetz, O.-M. (1984) L'éthologie au service de la systématique: l'exemple des *Trapezia* (Crustacés Décapodes, Brachyoures). *Oceanis*, **10**, 123–130.

OIE (2003) *Manual of Diagnostic Tests for Aquatic Animals*, 2003. 4th edn. Office International des Épizooties, Paris, France.

Olson, J.A. (1993) Vitamin A and carotenoids as antioxidants in a physiological context. *Journal of Nutritional Science and Vitaminology*, **39**, S57–S65.

Olson, R.R. & Olson, M.H. (1989) Food limitation of planktotrophic marine invertebrate larvae: does it control recruitment success? *Annual Review of Ecology and Systematics*, **20**, 225–247.

Olson, V.A. & Owens, I.P.F. (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution*, **13**, 510–514.

Omori, K., Yanagisawa, Y. & Hori, N. (1994) Life history of the caridean shrimp *Periclimenes ornatus* Bruce associated with a sea anemone in southwest Japan. *Journal of Crustacean Biology*, **14**, 132–145.

Orians, G.H. & King, C.E. (1964) Shell selection and invasion rates of some Pacific hermit crabs. *Pacific Science*, **18**, 297–306.

Otsu, T. (1963) Bihormonal control of sexual cycle in the fresh water crab, *Potamon dehaani*. *Embryologica*, **8**, 1–20.

Paffenhöfer, G.-A. & Knowles, S.C. (1978) Feeding of marine planktonic copepods on mixed phytoplankton. *Marine Biology*, **48**, 143–152.

Palacios, E. & Racotta, I.S. (2007) Salinity stress test and its relation to future performance and different physiological responses in shrimp postlarvae. *Aquaculture*, **268**, 123–135.

Palacios, E., Ibarra, A.M., Ramirez, J.L., Portillo, G. & Racotta, I.S. (1998) Biochemical composition of eggs and nauplii in White Pacific Shrimp, *Penaeus vannamei* (Boone), in relation to the physiological condition of spawners in a commercial hatchery. *Aquaculture Research*, **29**, 183–189.

Palacios, E., Perez-Rostro, C.I., Ramirez, J.L., Ibarra, A.M. & Racotta, I.S. (1999) Reproductive exhaustion in shrimp *Penaeus vannamei* reflected in larval biochemical composition, survival and growth. *Aquaculture*, **171**, 309–321.

Palacios, E., Racotta, I.S., Marty, Y. & Samain, J.F. (2001a) Lipid composition during embryogenesis and early larval development in shrimp (*Penaeus vannamei*). *European Aquaculture Society, Special Publication*, **30**, 453–456.

Palacios, E., Racotta, I.S., Heras, H., Marty, Y., Moal, J. & Samain, J.F. (2001b) The relation between lipid and fatty acid composition of eggs and larval survival in white pacific shrimp (*Penaeus vannamei*, Boone, 1931). *Aquaculture International*, **9**, 531–543.

Palma, A.T. & Steneck, R.S. (2001) Does variable coloration in juvenile marine crabs reduce risk of visual predation? *Ecology*, **82**, 2961–2967.

Palma, A.T., Orrego, C. & Arrigadas, M. (2003) Crypsis in early benthic phases of brachyuran decapod crustaceans in central Chile. *Revista Chilena de Historia Natural*, **76**, 149–156.

Palmer, P.J., Burke, M.J., Palmer, C.J. & Burke, J.B. (2007) Developments in controlled green-water larval culture technologies for estuarine fishes in Queensland, Australia and elsewhere. *Aquaculture*, **272**, 1–21.

Palmtag, M.R. & Holt, G.J. (2001) *Captive rearing of fire shrimp* (Lysmata debelius). Texas Sea Grant College Program Research Report, Texas A&M University, Galveston, Texas.

Palmtag, M.R. & Holt, G.J. (2007) Experimental studies to evaluate larval survival of the fire shrimp, *Lysmata debelius*, to the juvenile stage. *Journal of the World Aquaculture Society*, **38**, 102–113.

Palmtag, M.R., Holt, G.J. & Faulk, C.K. (2006) Highly unsaturated fatty acid composition of rotifer (*Brachionus plicatilis*) and *Artemia* fed various enrichments. *Journal of the World Aquaculture Society*, **37**, 126–131.

Palomar, N.E., Juinio-Meñez, M.A. & Karplus, I. (2004) Feeding habits of the burrowing shrimp *Alpheus macellarius*. *Journal of the Marine Biological Association of the United Kingdom*, **84**, 1199–1202.

Palomar, N.E., Juinio-Meñez, M.A. & Karplus, I. (2005) Behavior of the burrowing shrimp *Alpheus macellarius* in varying gravel substrate conditions. *Journal of Ethology*, **23**, 173–180.

Pangantihon-Kühlmann, M.P., Millamena, O. & Chern, Y. (1998) Effect of dietary astaxanthin and vitamin A on the reproductive performance of *Penaeus monodon* broodstock. *Aquatic Living Resources*, **11**, 403–409.

Panouse, J.B. (1943) Influence de l' ablation du pédoncle oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. *Comptes Rendus de l'Académie des Sciences, Paris*, **218**, 293–294.

Papandroulakis, N., Divanach, P., Anatasiadis, P. & Kentouri, M. (2001) The pseudo-green water technique for intensive rearing of sea bream (*Sparus aurata*) larvae. *Aquaculture International*, **9**, 205–216.

Papandroulakis, N., Kentouri, M., Maingot, E. & Divanach, P. (2004) Mesocosm: a reliable technology for larval rearing of *Diplodus puntazzo* and *Diplodus sargus sargus*. *Aquaculture International*, **12**, 345–355.

Parker, R.B. (1974) Probiotics. The other half of the antibiotics story. *Animal Nutrition and Health*, **29**, 4–8.

Paschke, K.A., Gebauer, P., Buchhola, F. & Anger, K. (2004) Seasonal variation in starvation resistance of early larval North Sea shrimp *Crangon crangon* (Decapoda, Crangonidae). *Marine Ecology Progress Series*, **279**, 183–191.

Paterson, B.D., Spanoghe, P.T., Davidson, G.W., Hosking, W., Nottingham, S., Jussila, J. & Evans, L.H. (2005) Predicting survival of western rock lobsters *Panulirus cygnus* using discriminant analysis of haemolymph parameters taken immediately following simulated handling treatments. *New Zealand Journal of Marine and Freshwater Research*, **39**, 1129–1143.

Patnaik, S., Samocha, T.M., Davis, D.A., Bullis, R.A. & Browdy, C.L. (2006) The use of HUFA-rich algal meals in diets for *Litopenaeus vannamei*. *Aquaculture Nutrition*, **12**, 395–401.

Patton, W.K. (1974) Community structure among the animals inhabiting the coral *Pocillopora damicornis* at Heron Island, Australia. In: *Symbiosis and the Sea* (ed. W.B. Vernberg) pp. 219–243. University of South Carolina Press, Columbia, USA.

Paul, A.J. & Paul, J.M. (1992) Second clutch viability of *Chionoecetes bairdi* Rathbun (Decapoda: Majidae) inseminated only at the maturity molt. *Journal of Crustacean Biology*, **12**, 438–441.

Paul, A.J., Paul, J.M., Shoemaker, P.A. & Feder, H.M. (1979) Prey concentrations and feeding response in laboratory-reared stage-one zoeae of king crab, snow crab, and pink shrimp. *Transactions of the American Fisheries Society*, **108**, 440–443.

Paula, J. & Cartaxana, A. (1991) Complete larval development of the spider crab *Stenorhynchus lanceolatus* (Brullé, 1837) (Decapoda, Brachyura, Majidae), reared in the laboratory. *Crustaceana*, **60**, 113–122.

Paula, J. & dos Santos, A. (2000) Larval and early post-larval stages of the crab, *Xantho pilipes* A. Milne-Edwards (Crustacea, Decapoda, Xanthidae), reared under laboratory conditions. *Invertebrate Reproduction and Development*, **38**, 253–264.

Paula, J. & Hartnoll, R.G. (1989) The larval and post-larval development of *Percnon gibbesi* (Crustacea, Brachyura, Grapsidae) and the identity of the larval genus *Pluteocaris*. *Journal of Zoology, London*, **218**, 17–37.

Pawlic, J.R. (1992) Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology Annual Review*, **30**, 273–335.

Payen, G.G. (1983) Endocrine regulation of male genital development in Malacostraca. *American Zoology*, **23**, 951.

Payne, M.F. & Rippingale, R.J. (2000) Evaluation of diets for culture of the calanoid copepod *Gladioferens imparipes*. *Aquaculture*, **187**, 85–96.

Payne, M.F. & Rippingale, R.J. (2001) Intensive cultivation of the calanoid copepod *Gladioferens imparipes*. *Aquaculture*, **201**, 329–342.

Payne, M.S., Hall, M.R., Bannister, R., Sly, L. & Bourne, D.G. (2006) Microbial diversity within the water column of a larval rearing system for the ornate rock lobster (*Panulirus ornatus*). *Aquaculture*, **258**, 80–90.

Pechenik, J.A. (1990) Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? *Ophelia*, **32**, 63–94.

Pechenik, J.A. (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series*, **177**, 269–297.

Peck, M.A. & Holste, L. (2006) Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): optimizing intensive cultures. *Aquaculture*, **255**, 341–350.

Peixoto, S.M., Wasielesky, W., Jr, D'Incao, F. & Cavalli, R.O. (2003) Comparison of the reproductive performance of similarly-sized wild and captive *Farfantepenaeus paulensis*. *Journal of the World Aquaculture Society*, **34**, 50–56.

Penha-Lopes, G., Rhyne, A.L., Lin, J. & Narciso, L. (2005) The larval rearing of the marine ornamental crab, *Mithraculus forceps* (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae). *Aquaculture Research*, **36**, 1313–1321.

Penha-Lopes, G., Rhyne, A.L., Lin, J. & Narciso, L., (2006a) Effects of temperature, stocking density and diet on the growth and survival of juvenile *Mithraculus forceps* (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae). *Aquaculture Research*, **37**, 398–408.

Penha-Lopes, G., Rhyne, A.L., Figueiredo, J., Lin, J. & Narciso, L. (2006b) Can larvae produced from stored sperm in the ornamental crab *Mithraculus forceps* (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae) be used in aquaculture? *Aquaculture*, **257**, 282–286.

Perez, M.F. & Sulkin, S.D. (2005) The palatability of autotrophic dinoflagellates in larval crabs. *Marine Biology*, **146**, 771–780.

Perez-Velazquez, M., González-Félix, M.L., Lawrence, A.L., Bray, W.A. & Gatlin, D.M. III (2003) Dietary effects on sperm quality of *Litopenaeus vannamei* broodstock. *Journal of the World Aquaculture Society*, **34**, 92–98.

Perry, H., Trigg, C., Larsen, K., Freeman, J., Erickson, M. & Henry, R. (2001) Calcium concentration in seawater and exoskeletal calcification in the blue crab, *Callinectes sapidus*. *Aquaculture*, **198**, 197–208.

Pet, J. & Pet-Soede, L. (1999) A note on cyanide fishing in Indonesia. *Secretariat of the Pacific Community Live Reef Fish Information Bulletin*, **5**, 21–22.

Petit, H., Sance, S., Négre-Sadargues, G., Castillo, R. & Trilles, J.-P. (1991) Ontogeny of carotenoid metabolism in the prawn *Penaeus japonicus* Bate (1888) (Crustacea: Penaeidae). A qualitative approach. *Comparative Biochemistry and Physiology, Part B*, **99**, 667–671.

Petit, H., Négre-Sadargues, G., Castillo, R. & Trilles, J.-P. (1997) The effects of dietary astaxanthin on growth and moulting cycle of postlarval stages of the prawn, *Penaeus japonicus* (Crustacea, Decapoda) — premiers resultats. *Comparative Biochemistry and Physiology, Part A*, **117**, 539–544.

Phillips, B.F. & Evans, L.H. (1997) Aquaculture and stock enhancement of lobsters: report from a workshop. *Marine and Freshwater Research*, **48**, 899–902.

Phillips, B.F. & Sastry, A.N. (1980) Larval ecology. In: *The Biology and Management of Lobsters*, Vol. 2 (eds J.S. Cobb & B.F. Phillips) pp. 11–57. Academic Press, New York, USA.

Phillips, B.F., Melville-Smith, R., Rossbach, M., Cheng, Y.W., Caputi, N., Thompson, A.W., Mills, D. & Crear, B. (2003) Rock lobster enhancement and aquaculture subprogram: towards establishing techniques for large scale harvesting of pueruli and obtaining a better understanding of mortality rates. *Western Australian Marine Research Laboratories Report 144*. North Beach, Western Australia, Australia.

Phleger, C.F., Nichols, P.D. & Virtue, P. (1998) Lipids and trophodynamics of Antarctic zooplankton. *Comparative Biochemistry and Physiology, Part B*, **120**, 311–323.

Pike, R.B. & Williamson, D.I. (1960) Larvae of decapod Crustacea of the families Diogenidae and Paguridae from the Bay of Naples. *Pubblicazione de la Stazione Zoologica di Napoli*, **31**, 493–552.

Pipitone, C., Badalamenti, F. & Sparrow, A. (2001) Contribution to the knowledge of *Percnon gibbesi* (Decapoda, Grapsidae), an exotic species spreading rapidly in Sicilian waters. *Crustaceana*, **74**, 1009–1017.

Pittet, A.O., Ellis, J.C. & Lee, P.G. (1996) Methodology for the identification and quantitative measurement of chemical stimulants for penaeid shrimp. *Aquaculture Nutrition*, **2**, 175–182.

Pohle, G. W. and Marques, F. (2000) Larval stages of *Paradasygyius depressus* (Bell, 1835) (Crustacea: Decapoda: Brachyura: Majidae) and a phylogenetic analysis for 21 genera of Majidae. *Proceedings of the Biological Society of Washington*, **113**, 739–760.

Pomeroy, R.S. & Balboa, C. (2004) The financial feasibility of small-scale marine ornamental aquaculture in the Philippines. *Asian Fisheries Science*, **17**, 365–376.

Pond, D., Dixon, D. & Sargent, J. (1997) Wax-ester reserves facilitate dispersal of hydrothermal vent shrimps. *Marine Ecology Progress Series*, **146**, 289–290.

Pontes, C.S., Arruda, M.F., Menezes, A.A.L. & Lima, P.P. (2006) Daily activity pattern of the marine shrimp *Litopenaeus vannamei* (Boone 1931) juveniles under laboratory conditions. *Aquaculture Research*, **37**, 1001–1006.

Poulin, E., Boletzky, S.V. & Feral, J.-P. (2001) Combined ecological factors permit classification of developmental patterns in benthic marine invertebrates: a discussion note. *Journal of Experimental Marine Biology and Ecology*, **257**, 109–115.

Poulin, R. & Vickery, W.L. (1995) Cleaning symbiosis as an evolutionary game: to cheat or not to cheat? *Journal of Theoretical Biology*, **64**, 52–69.

Powell, C.B. (1979) Suppression of larval development in the African freshwater shrimp *Desmocaris trispinosa*. *Crustaceana*, **Supplement 5**, 185–194.

Pratchett, M.S. (2001) Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the western Pacific. *Marine Ecology Progress Series*, **214**, 111–119.

Pratchett, M.S. (2005) Dynamics of an outbreak population of *Acanthaster planci* at Lizard Island, northern Great Barrier Reef (1995–1999). *Coral Reefs*, **24**, 453–462.

Pratchett, M.S., Vytopil, E. & Parks, P. (2000) Coral crabs influence the feeding patterns of crown-of-thorns starfish. *Coral Reefs*, **19**, 36.

Preston, E.M. (1971) Niche overlap and competition among five sympatric congeneric species of xanthid crabs. PhD thesis, University of Hawaii, Honolulu.

Preston, J.L. (1978) Communication systems and social interactions in a goby–shrimp symbiosis. *Animal Behaviour*, **26**, 791–802.

Preston, N.P., Burford, M.A., Coman, F.E. & Rothlisberg, P.C. (1992) Natural diet of larval *Penaeus merguiensis* (Decapoda: Penaeidae) and its effect on survival. *Marine Biology*, **113**, 181–191.

Preston, N.P., Crocos, P.J., Keys, S.J., Coman, G.J. & Koenig, R. (2004) Comparative growth of selected and non-selected Kuruma shrimp *Penaeus (Marsupenaeus) japonicus* in commercial farm ponds; implications for broodstock production. *Aquaculture*, **231**, 73–82.

Provenzano, A.J., Jr. (1962) The larval development of *Calcinus tibicen* (Herbst) (Crustacea, Anomura) in the laboratory. *Biological Bulletin*, **123**, 179–202.

Puccio, V., Relini, M., Azzurro, E. & Orsi Relini, L. (2006) Feeding habits of *Percnon gibbesi* (H. Milne Edwards, 1853) in the Sicily Strait. *Hydrobiologia*, **557**, 79–84.

Quackenbush, L.S. (1989) Yolk protein production in the marine shrimp *Penaeus vannamei*. *Journal of Crustacean Biology*, **9**, 509–516.

Rabbani, A.G. & Zeng, C. (2005) Effects of tank colour on larval survival and development of mud crab, *Scylla serrata* (Forskål). *Aquaculture Research*, **36**, 1112–1119.

Racotta, I.S., Palacios, E. & Ibarra, A.M. (2003) Shrimp larval quality in relation to broodstock condition. *Aquaculture*, **227**, 107–130.

Racotta, I.S., Palacios, E., Hernández-Herrera, R., Bonilla, A., Pérez-Rostro, C.I. & Ramírez, J.L. (2004) Criteria for assessing larval and postlarval quality of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). *Aquaculture*, **233**, 181–195.

Radford, C.A., Marsden, I.D., Davison, W. & Taylor, H.H. (2005) Haemolymph glucose concentrations of juvenile rock lobsters, *Jasus edwardsii*, feeding on different carbohydrate diets. *Comparative Biochemistry and Physiology, Part A*, **140**, 241–249.

Rainbow, P.S. (1974) The demonstration of chemosensory food detection in *Hymenocera picta* Dana. *African Journal of Hydrobiology and Fisheries*, **3**, 183–191.

Ranjeet, K. & Kurup, B.M. (2002) Management strategies associating batch-graded and size-graded postlarvae can reduce heterogeneous individual growth in *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*, **33**, 1221–1231.

Rasmussen, R.S. & Morrissey, M.T. (2007) Biotechnology in aquaculture: transgenics and polyploidy. *Comprehensive Reviews in Food Science and Food Safety*, **6**, 2–16.

Ravid, T., Tietz, A., Khayat, M., Boehm, E., Michelis, R. & Lubzens, E. (1999) Lipid accumulation in the ovaries of a marine shrimp *Penaeus semisulcatus* (De Haan). *Journal of Experimental Biology*, **202**, 1819–1829.

Reddy, H.R.V., Ganapathi Naik, M. & Annappaswamy, T.S. (1999) Evaluation of the dietary essentiality of vitamins for *Penaeus monodon*. *Aquaculture Nutrition*, **5**, 267–275.

Reese, E.S. (1963) The behavioral mechanisms underlying shell selection by hermit crabs. *Behaviour*, **21**, 78–126.

Reese, E.S. (1964) Ethology and marine zoology. *Oceanography and Marine Biology*, **2**, 455–488.

Reese, E.S. (1968) Shell use; an adaptation for emigration from the sea by the coconut crab. *Science*, **161**, 385–386.

Regunathan, C. & Wesley, S.G. (2006) Pigment deficiency correction in shrimp broodstock using *Spirulina* as a carotenoid source. *Aquaculture Nutrition*, **12**, 425–432.

Relini, M., Orsi, L., Puccio, V. & Azzurro, E. (2000) The exotic crab *Percnon gibbesi* (H. Milne Edwards, 1853) in the Central Mediterranean. *Scientia Marina*, **64**, 337–340.

Rengpipat, S., Tunyamum, A., Fast, A.W., Piyatiratitivoraku, S. & Menasveta, P. (2003) Enhanced growth and resistance to vibrio challenge in pond-reared black tiger shrimp *Penaeus monodon* fed a *Bacillus* probiotic. *Diseases of Aquatic Organisms*, **55**, 169–173.

Rhyne, A.L. & Lin, J. (2004) Effects of different diets on larval development in a peppermint shrimp (*Lysmata* sp. (Risso)). *Aquaculture Research*, **35**, 1179–1185.

Rhyne, A.L. & Lin, J. (2006) A western peppermint shrimp complex: redescription of *Lysmata wurdemanni* (Gibbes), description of four new species and remarks on *L. rathbunae* Chace (Crustacea: Decapoda: Hippolytidae). *Bulletin of Marine Science*, **79**, 165–204.

Rhyne, A.J., Lin, J., Calado, R. & Turingan, R. (2001) Improvements in marine ornamentals shrimp culture: high speed video analysis of feeding kinematics in dietary study. *Marine Ornamentals 2001. 'Collection, Culture and Conservation'. Orlando, Florida, USA. Book of Abstracts*, 29–30.

Rhyne, A.L., Lin, J. & Deal, K. (2004) Biological control of aquarium pest anemone *Aiptasia pallida* (Verrill) by peppermint shrimp *Lysmata*. *Journal of Shellfish Research*, **23**, 227–229.

Rhyne, A.L., Penha-Lopesa, G. & Lin, J. (2005) Growth, development, and survival of larval *Mithraculus sculptus* (Lamark) and *Mithraculus forceps* (A. Milne Edwards) (Decapoda: Brachyura: Majidae): economically important marine ornamental crabs. *Aquaculture*, **245**, 183–191.

Rhyne, A.L., Fujita, Y. & Calado, R. (2006) Larval development and first crab of *Mithraculus sculptus* (Decapoda: Brachyura: Majoidea: Mithracidae) described from

laboratory-reared material. *Journal of the Marine Biological Association of the United Kingdom*, **86**, 1133–1147.

Richardson, L.B., Burton, D.T., Helz, G.R. & Rhoderick, J.C. (1981) Residual oxidant decay and bromate formation in chlorinated and ozonated sea-water. *Water Research*, **15**, 1067–1074.

Rimmer, D.W. & Phillips, B.F. (1979) Diurnal migration and vertical distribution of phyllosoma larvae of the western rock lobster *Panulirus cygnus*. *Marine Biology*, **54**, 109–124.

Ritar, A.J. (2001) The experimental culture of phyllosoma larvae of southern rock lobster (*Jasus edwardsii*) in a flow-through system. *Aquacultural Engineering*, **24**, 149–156.

Ritar, A.J., Smith, G.G., Dunstan, G.A., Brown, M.R. & Hart, P.R. (2003) Artemia prey size and mode of presentation: effects on the survival and growth of phyllosoma larvae of southern rock lobster (*Jasus edwardsii*). *Aquaculture International*, **11**, 163–182.

Ritar, A.J., Smith, G.G. & Thomas, C.W. (2006) Ozonation of seawater improves the survival of larval southern rock lobster, *Jasus edwardsii*, in culture from egg to juvenile. *Aquaculture*, **261**, 1014–1025.

Ritz, D.A. (1972) Behavioural response to light of the newly hatched phyllosoma larvae of *Panulirus longipes cygnus* George (Crustacea: Decapoda: Palinuridae). *Journal of Experimental Marine Biology and Ecology*, **10**, 105–114.

Robertson, P.B. (1969) Phyllosoma larvae of a palinurid lobster, *Justitia longimana* (H. Milne Edwards), from the Western Atlantic. *Bulletin of Marine Science*, **19**, 922–944.

Robinson, C.B., Samocha, T.M., Fox, J.M., Gandy, R.L. & McKee, D.A. (2005) The use of inert artificial commercial food sources as replacements of traditional live food items in the culture of larval shrimp, *Farfantepenaeus aztecus*. *Aquaculture*, **245**, 135–147.

Rodrigues, C.C.B., Rodrigues, J.B.R. & Moschen, S. (1998) The effects of sunlight and tank colour on the development and post-larvae production of *Macrobrachium rosenbergii*. *Anais da Aquicultura do Brasil*, **2**, 423–435.

Rodriguez, E.M., Triño, A.T. & Mingawa, M. (2003) Diet and harvesting regimen for the production of mud crab *Scylla olivacea* in brackish water ponds. *Fisheries Science*, **69**, 37–42.

Rodríguez, R.A. & Epifanio, C.E. (2000) Multiple cues for induction of metamorphosis in larvae of the common mud crab *Panopeus herbstii*. *Marine Ecology Progress Series*, **195**, 221–229.

Rombaut, G., Dhert, P., Vandenberghe, J., Verschuere, L., Sorgeloos, P. & Verstraete, W. (1999) Selection of bacteria enhancing the growth rate of axenically hatched rotifers (*Brachionus plicatilis*). *Aquaculture*, **176**, 195–207.

Ronquillo, J.D. & Saisho, T. (1997) Larval development of *Metapenaeopsis barbata* (de Haan, 1844) (Crustacea: Decapoda: Paneidae). *Marine and Freshwater Research*, **48**, 401–414.

Rosa, R., Morais, S., Calado, R., Narciso, L. & Nunes, M.L. (2003) Biochemical changes during the embryonic development of Norway lobster, *Nephrops norvegicus*. *Aquaculture*, **211**, 507–522.

Rosa, R., Calado, R., Andrade, A.M., Narciso, L. & Nunes, M.L. (2005) Changes in amino acids and lipids during embryogenesis of European lobster, *Homarus gammarus* (Crustacea: Decapoda). *Comparative Biochemistry and Physiology, Part B*, **140**, 241–249.

Rosa, R., Calado, R., Narciso, L. & Nunes, M.L. (2007) Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies and habitats: a fatty acid approach. *Marine Biology*, **151**, 935–947.

Rosas, C., Cuzon, G., Gaxiola, G., Arena, L., Lemaire, P., Soyez, C. & Van Wormhoult, A. (2000) Influence of dietary carbohydrate on the metabolism of juvenile *Litopenaeus stylirostris*. *Journal of Experimental Marine Biology and Ecology*, **249**, 181–198.

Rothlisberg, P.C. (1998) Aspects of penaeid biology and ecology of relevance to aquaculture; a review. *Aquaculture*, **164**, 49–65.

Roubal, F.R., Quartararo, N. & West, A. (1992) Infection of captive *Pagrus auratus* (Bloch & Schneider) by the monogenean, *Anoplodiscus cirrusspiralis* Roubal, Armitage & Rohde (Anoplodiscidae) in Australia. *Journal of Fish Diseases*, **15**, 409–415.

Rubec, P., Cruz, F., Pratt, V., Oellers, R. & Lallo, F. (2000) Cyanide-free, netcaught fish for the marine aquarium trade. *Secretariat of the Pacific Community Live Reef Fish Bulletin*, **7**, 28–34.

Rueter, J. & Johnson, R. (1995) The use of ozone to improve solids removal during disinfection. *Aquacultural Engineering*, **14**, 123–141.

Rufino, M. & Jones, D.A. (2001a) Binary individual recognition in *Lysmata debelius* (Bruce, 1983) (Crustacea: Hippolytidae) under laboratory conditions. *Journal of Crustacean Biology*, **21**, 388–392.

Rufino, M.M. & Jones, D.A. (2001b) Observations on the function of the fifth pereiopod in late stage larvae of *Lysmata debelius* (Decapoda, Hippolytidae). *Crustaceana*, **74**, 977–990.

Ruiz-Carus, R., Jr, Matheson, R.E., Jr, Roberts, D.E. & Whitfield, P.E. (2006) The western Pacific red lionfish, *Pterois volitans* (Scorpaenidae), in Florida: evidence for reproduction and parasitism in the first exotic marine fish established in state waters. *Biological Conservation*, **128**, 384–390.

Ruscoe, I.M., Williams, G.R. & Shelley, C.C. (2004) Limiting the use of rotifers to the first zoeal stage in mud crab (*Scylla serrata* Forskål) larval rearing. *Aquaculture*, **231**, 517–527.

Sadovy, Y. & Shapiro, D.Y. (1987) Criteria for the diagnosis of hermaphroditism in fishes. *Copeia*, **1987**, 136–156.

Sagi, A. & Aflalo, E.D. (2005) The androgenic gland and monosex culture of freshwater prawn *Macrobrachium rosenbergii* (De Man): a biotechnological perspective. *Aquaculture Research*, **36**, 231–237.

Sagi, A. & Cohen, D. (1990) Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males. *World Aquaculture*, **21**, 87–90.

Sagi, A. & Khalaila, I. (2001) The crustacean androgen: a hormone in an isopod and androgenic activity in decapods. *American Zoologist*, **41**, 477–484.

Sagi, A., Milstein, A., Eran,Y., Joseph, D., Khalaila, I., Abdu, U., Harpaz, S. & Karplus, I. (1997a) Culture of the Australian redclaw crayfish (*Cherax quadricarinatus*) in Israel, II. Second growout season of overwintered populations. *Israeli Journal of Aquaculture — Bamidgeh*, **49**, 222–229.

Sagi, A., Snir, E. & Khalaila, I. (1997b) Sexual differentiation in decapod crustaceans: role of the androgenic gland. *Invertebrate Reproduction and Development*, **31**, 55–61.

Sahul Hameed, A.S. & Balasubramanian, G. (2000) Antibiotic resistance in bacteria isolated from *Artemia* nauplii and efficacy of formaldehyde to control bacterial load. *Aquaculture*, **183**, 195–205.

Sainte-Marie, B. & Carriere, C. (1995) Fertilization of the second clutch of eggs of snow crab, *Chionoecetes opilio*, from females mated once or twice after their molt to maturity. *Fishery Bulletin*, **93**, 759–764.

Sainte-Marie, B. & Lafrance, M. (2002) Growth and survival of recently settled snowcrab *Chionoecetes opilio* in relation to intra- and intercohort competition and cannibalism: a laboratory study. *Marine Ecology Progress Series*, **244**, 191–203.

Saito, T. & Konishi, K. (1999) Direct development in the sponge-associated deep-sea shrimp *Spongicola japonica* (Decapoda: Spongicolidae). *Journal of Crustacean Biology*, **19**, 46–52.

Saito, T. & Takeda, M. (2003) Phylogeny of the family Spongicolidae (Crustacea: Stenopodidea): evolutionary trend from shallow-water free-living to deep water sponge-associated habitat. *Journal of the Marine Biological Association of the United Kingdom*, **83**, 119–131.

Samocha, T.M., Guajardo, H., Lawrence, A.L., Castille, F.L., Speed, M., McKee, D.A. & Page, K.I. (1998) A simple stress test for *Penaeus vannamei* postlarvae. *Aquaculture*, **165**, 233–242.

Sandifer, P.A. & Smith, T.I.J. (1979) Possible significance of variation in the larval development of palaemonid shrimp. *Journal of Experimental Marine Biology and Ecology*, **39**, 55–64.

Sangha, R.S., Puello Cruz, A.C., Chavez-Sanchez, M.C. & Jones, D.A. (2000) Survival and growth of *Litopenaeus vannamei* (Boone) larvae fed a single dose of live algae and artificial diets with supplements. *Aquaculture Research*, **31**, 683–689.

Sankolli, K.N. & Kewalramani, H.G. (1962) Larval development of *Saron marmoratus* (Olivier), in the laboratory. *Journal of the Marine Biological Association of India*, **4**, 106–120.

Sargent, R.C. & Wagenbach, G.E. (1975) Cleaning behavior of the shrimp, *Periclimenes anthophilus* Holthius and Eibl-Eibesfeldt (Crustacea: Decapoda: Natantia). *Bulletin of Marine Science*, **25**, 466–472.

Sarver, D. (1979) Larval culture of the shrimp *Thor amboinensis* (De Man, 1888) with reference to its symbiosis with the anemone *Antheopsis papillosa* (Kwietniwski, 1898). *Crustaceana*, **5**, 176–178.

Sastry, A.N. (1970) Culture of brachyuran crab larvae using re-circulating sea water system in the laboratory. *Helgolander Meeresunters*, **20**, 406–416.

Sastry, A.N. & Zeitlin-Hale, L. (1977) Survival of communally reared larval and juvenile lobsters, *Homarus americanus*. *Marine Biology*, **39**, 297–303.

Sastry, B.N., DeLosReyes, A.A., Jr, Rusch, K.A. & Malone, R.F. (1999) Nitrification performance of a bubble-washed bead filter for combined solids removal and biological filtration in a recirculating aquaculture system. *Aquacultural Engineering*, **19**, 105–117.

Sato, Y. & Seno, H. (2006) A mathematical consideration for the optimal shell change of hermit crab. *Journal of Theoretical Biology*, **240**, 14–23.

Schipp, G.R (2006) The use of calanoid copepods in semi-intensive, tropical marine fish larviculture. In: *Avances en Nutrición Acuicola VIII. Memorías del Octavo Simposium Internacional de Nutrición Acuícola* (eds L.E.C. Suárez, D.R. Marie, M.T. Salazar, M.G.N. López, D.A.V. Cavazoz, A.C.P. Cruz & A.G. Ortega) pp. 84–94. Monterrey, Nuevo Léon, México.

Schipp, G.R., Bosmans, J.M.P. & Marshall, A.J. (1999) A method for hatchery culture of tropical calanoid copepods, *Acartia* spp. *Aquaculture*, **174**, 81–88.

Schlacher, T.A., Stark, J. & Fischer, A.B.P. (2007) Evaluation of artificial light regimes and substrate types for aquaria propagation of the staghorn coral *Acropora solitaryensis*. *Aquaculture*, **269**, 278–289.

Schmitz, B. (2001) Sound production in Crustacea with special reference to the Alpheidae. In *Physiology of the Crustacean Nervous System* (ed. K. Wiese) pp. 521–533. Springer Verlag, Berlin.

Schmitz, B. & Herberholz, J. (1998) Snapping behaviour in intraspecific agonistic encounters in the snapping shrimp (*Alpheus heterochaelis*). *Journal of Biosciences*, **23**, 623–632.

Scholtz, G. (2000) Evolution of the nauplius stage in malacostracan crustaceans. *Journal of Zoological Systematics & Evolutionary Research*, **38**, 175–187.

Sedgwick, R.W. (1979) Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* De Man. *Aquaculture*, **16**, 7–30.

Seibt, U. (1973) Sense of smell and pair-bond in *Hymenocera picta* Dana. *Micronesia*, **9**, 231–236.

Seibt, U. (1974) Mechanismen und Sinnesleistungen für den Paarzusammenhalt bei der Garnele *Hymenocera picta* Dana. *Zeitschrift für Tierpsychologie*, **35**, 337–351.

Seibt, U. & Wickler, W. (1979) The biological significance of the pair-bond in the shrimp *Hymenocera picta*. *Zeitschrift für Tierpsychologie*, **50**, 166–179.

Sekine, S., Shima, Y., Fushimi, H. & Nonaka, M. (2000) Larval period and molting in the Japanese spiny lobster *Panulirus japonicus* under laboratory conditions. *Fisheries Science*, **66**, 19–24.

Sellars, M.J., Coman, G.J. & Morehead, D.T. (2005) Tolerance of *Penaeus* (*Marsupenaeus*) *japonicus* embryos to ozone disinfection. *Aquaculture*, **245**, 111–119.

Semmens, B.X., Buhle, E.R., Salomon, A.K. & Pattengill-Semmens, C.V. (2004) A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. *Marine Ecology Progress Series*, **266**, 239–244.

Seridji, R. (1990) Description of some planktonic larval stages of *Stenopus spinosus* Risso, 1826: notes on the genus and systematic position of the Stenopodidea as revealed by larval characters. *Scientia Marina*, **54**, 293–303.

Shafland, P.L. (1996) Exotic fishes of Florida — 1994. *Reviews in Fisheries Science*, **4**, 101–122.

Sheen, S.-S. (2000) Dietary cholesterol requirement of juvenile mud crab *Scylla serrata*. *Aquaculture*, **189**, 277–285.

Sheen S.-S. & Wu S.-W. (1999) The effects of dietary lipid levels on the growth response of juvenile mud crab *Scylla serrata*. *Aquaculture*, **175**, 143–153.

Shewbart, K.L., Mies, W.L. & Ludwig, P.D. (1972) Identification and quantitative analysis of the amino acids present in protein of the brown shrimp *Penaeus aztecus*. *Marine Biology*, **16**, 64–67.

Shieh, H.S. (1969) The biosynthesis of phospholipids in the lobster *Homarus americanus*. *Comparative Biochemistry and Physiology*, **30**, 679–684.

Shimek, R.L. (2004) *Marine Invertebrates —500+ Essential-to-Know Aquarium Species.* T.F.H. Publications Inc., Neptune City, New Jersey, USA.

Siddiqui, F.A., McLaughlin, P.A. & Crain, J.A. (1993) Larval development of the hermit crab *Clibanarius albidigitus* (Crustacea: Anomura: Diogenidae) reared under laboratory conditions. *Marine Biology*, **116**, 603–613.

Silliman, B.R., Layman, C.A. & Altieri, A.H. (2003) Symbiosis between an alpheid shrimp and a xanthoid crab in salt marshes of Mid-Atlantic States, USA. *Journal of Crustacean Biology*, **23**, 876–879.

Simões, F., Ribeiro, F. & Jones, D.A. (1998) The effect of diet on the reproductive performance of marine cleaner shrimps *L. debelius* (Bruce, 1983) and *Lysmata amboinensis* (de Man, 1888) (Caridea, Hippolytidae) in captivity. *Aquaculture 98 Book of Abstracts, Las Vegas, Nevada, USA*, 497.

Simões, F., Ribeiro, F. & Jones, D.A. (2002) Feeding early larval stages of fire shrimp *Lysmata debelius* (Caridea, Hippolytidae). *Aquaculture International*, **10**, 349–360.

Singh, I.S.B. & Philip, R. (1995) A simple device for the separation of weak larvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*, **26**, 225–227.

Skjermo, J. & Vadstein, O. (1999) Techniques for microbial control in intensive rearing of marine larvae. *Aquaculture*, **177**, 333–343.

Smith, D.M., Williams, K.C., Irvin, S., Barclay, M. & Tabret, S. (2003) Development of a pelleted feed for juvenile tropical spiny lobster (*Panulirus ornatus*): response to dietary protein and lipid. *Aquaculture Nutrition*, **9**, 231–237.

Smith, D.M., Tabret, S., Barclay, M. & Irvin, S. (2005) The efficacy of ingredients included in shrimp feeds to stimulate intake. *Aquaculture Nutrition*, **11**, 263–272.

Smith, G.G., Ritar, A.J., Thompson, P.A., Dunstan, G.A. & Brown, M.R. (2002a) The effect of embryo incubation temperature on indicators of larval viability in Stage I phyllosoma of the spiny lobster, *Jasus edwardsii*. *Aquaculture*, **209**, 157–167.

Smith, G.G., Ritar, A.J., Phleger, C.F., Nelson, M.M., Mooney, B., Nichols, P.D. & Hart, P.R. (2002b) Changes in gut content and composition of juvenile *Artemia* after oil enrichment and during starvation. *Aquaculture*, **208**, 137–158.

Smith, G.G., Ritar, A.J., Johnston, D. & Dunstan, G.A. (2004) Influence of diet on broodstock lipid and fatty acid composition and larval competency in the spiny lobster, *Jasus edwardsii*. *Aquaculture*, **233**, 451–475.

Smith, L.L., Fox, J.M., Treece, G.D. & McVey, J.P. (1993) Intensive larviculture techniques. In: *Handbook of Mariculture* (ed. J.P. McVey) pp. 153–172. CRC Press, Boca Raton, Florida, USA.

Sorgeloos, P., Coutteau, P., Dhert, P., Merchie, G. & Lavens, P. (1998) Use of brine shrimp, *Artemia* spp., in larval crustacean nutrition: a review. *Reviews in Fisheries Science*, **6**, 55–68.

Spitschakoff, T. (1912) *Lysmata seticaudata* Risso, als Beispiel eines echten Hermaphroditismus bei en Decapoden. *Zeitschrift für Wissenschaftliche Zoologie*, **100**, 190–209.

Spotte, S. (1996) Supply of regenerated nitrogen to sea anemones by their symbiotic shrimp. *Journal of Experimental Marine Biology and Ecology*, **198**, 27–36.

Spotte, S. (1998) "Cleaner" shrimps? *Helgoländer Meeresuntersuchungen*, **52**, 59–64.

Spotte, S. & Bubucis, P.M. (1997) Captive survivorship of the spotted anemone shrimp, *Periclimenes yucatanicus*. *Aquarium Sciences and Conservation*, **1**, 65–69.

Sprung, J. (1999) *Oceanographic Series — Corals: A Quick Reference Guide*. Ricordea Publishing, Miami, Florida, USA.

Sprung, J. (2001) *Oceanographic Series — Invertebrates: A Quick Reference Guide*. Ricordea Publishing, Miami, Florida, USA.

Sprung, J. (2002) *Algae: A Problem Solver*. Ricordea Publishing, Miami, Florida, USA.

Stachowicz, J.J. & Hay, M.E. (1996) Facultative mutualism between an herbivorous crab and a coralline alga: advantages of eating noxious seaweeds. *Oecologia*, **105**, 377–387.

Stachowicz, J.J. & Hay, M.E. (1999) Reduced mobility is associated with compensatory feeding and increased diet breadth of marine crabs. *Marine Ecology Progress Series*, **188**, 169–178.

Stephens, D.W. & Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press, Princeton, New Jersey, USA.

Stevens, B.G. (2003) Settlement, substratum preference, and survival of red king crab *Paralithodes camtschaticus* (Tilesius, 1815) glaucothoe on natural substrata in the laboratory. *Jounal of Experimental Marine Biology and Ecology*, **283**, 63–78.

Stevens, B.G. & Kittaka, R.A. (1998) Postlarval settling behaviour, substrate preference, and time to metamorphosis for red king crab *Paralithodes camtschaticus*. *Marine Ecology Progress Series*, **167**, 197–206.

Stevens, M. (2005) The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biological Reviews*, **80**, 573–588.

Stewart, H.L., Holbrook, S.J., Schmitt, R.J. & Brooks, A.J. (2006) Symbiotic crabs maintain coral health by clearing sediments. *Coral Reefs*, **25**, 609–615.

Stickney, A.P. & Perkins, H.C. (1981) Observations on the food of the larvae of the northern shrimp, *Pandalus borealis* Krøyer (Decapoda, Caridea). *Crustaceana*, **40**, 36–49.

Stolen, C. K. (1964) An analysis of the behavior of the cleaning shrimp, *Stenopus hispidus*. MS thesis, University of Hawaii, Honolulu.

Støttrup, J.G. (2000) The elusive copepods: their production and suitability in marine aquaculture. *Aquaculture Research*, **31**, 703–711.

Støttrup, J.G. (2006) A review on the status and progress in rearing copepods for marine larviculture. Advantages and disadvantages. Among calanoid, harpacticoid and cyclopoid copepods. In: *Avances en Nutrición Acuicola VIII. Memorías del Octavo Simposium Internacional de Nutrición Acuícola* (eds L.E.C. Suárez, D.R. Marie, M.T. Salazar, M.G.N. López, D.A.V. Cavazoz, A.C.P. Cruz & A.G. Ortega) pp. 62–83. Monterrey, Nuevo Léon, México.

Støttrup, J.G. & Jensen, J. (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. *Journal of Experimental Marine Biology and Ecology*, **141**, 87–105.

Støttrup, J.G., Shields, R., Gillespie, M., Gara, M.B., Sargent, L.R., Bell, J.G., Henderson, R.J., Tocher, D.R., Sutherland, R., Naas, T., Mangor Jensen, A., Naas, K., van der Meeren, T., Harboe, T., Sanchez, F.J., Sorgeloos, P., Dhert, P. & Fitzgerald, R. (1998) The production and use of copepods in larval rearing of halibut, turbot and cod. *Bulletin of the Aquaculture Association of Canada*, **4**, 41–46.

Støttrup, J.G., Bell, J.G. & Sargent, J.R. (1999) The fate of lipids during development and cold-storage of eggs in the laboratory-reared calanoid copepod, *Acartia tonsa* Dana, and in response to different algal diets. *Aquaculture*, **176**, 257–269.

Strathmann, R.R. (1987) Larval feeding. In: *Reproduction of Marine Invertebrates*, Vol. 9, (General Aspects: Seeking Unity in Diversity) (eds A.C. Giese, J.S. Pearse & V.B. Pearse) pp. 465–550. Blackwell Scientific Publications & The Boxwood Press, Pacific Grove, California, USA.

Strathmann, R.R. (1996) Are planktonic larvae of marine benthic invertebrates too scarce to compete within species? *Oceanologica Acta*, **19**, 399–407.

Stummer, L., Weller, J.A., Johnson, M.L. & Côté, I.M. (2004) Size and stripes: how fish clients recognize cleaners. *Animal Behaviour*, **68**, 145–150.

Suantika, G., Dhert, P., Rombaut, G., Vandenberghe, J., De Wolf, T. & Sorgeloos, P. (2001) The use of ozone in a high density recirculation system for rotifers. *Aquaculture*, **201**, 35–49.

Subrahmanyam, C. & Oppenheimer, C. (1970) The influence of feed levels on the growth of grooved penaeid shrimp in mariculture. *Proceedings of the World Mariculture Society*, **1**, 91–100.

Sudaryono, A., Tsvetnenko, E. & Evans, L.H. (1999) Evaluation of potential of lupin meal as an alternative to fish meal in juvenile *Penaeus monodon* diets. *Aquaculture Nutrition*, **5**, 277–285.

Sukenik, A. & Wahnon, R. (1991) Biochemical quality of marine unicellular algae with special emphasis on lipid composition: I. *Isochrysis galbana*. *Aquaculture*, **97**, 61–72.
Sukenik, A., Zmora, O. & Carmeli, Y. (1993) Biochemical quality of unicellular algae with special emphasis on lipid composition. II. *Nannochloropsis* sp. *Aquaculture*, **117**, 313–326.
Sulkin, S.D. (1975) The influence of light in the depth regulation of crab larvae. *Biological Bulletin*, **148**, 333–343.
Sulkin, S.D. (1978) Nutritional requirements during larval development of the portunid crab, *Callinectes sapidus* Rathbun. *Journal of Experimental Marine Biology and Ecology*, **34**, 29–41.
Sulkin, S.D. & Epifanio, C.E. (1975) Comparison of rotifers and other diets for rearing early larvae of the blue crab, *Callinectes sapidus* Rathbun. *Estuarine and Coastal Marine Science*, **3**, 109–113.
Sulkin, S.D. & McKeen, G. (1999) The significance of feeding history on the value of heterotrophic microzooplankton as prey for larval crabs. *Marine Ecology Progress Series*, **186**, 219–225.
Sulkin, S.D. & van Heukelem, W. (1980) Ecological and evolutionary significance of nutritional flexibility in planktotrophic larvae of the deep-sea red crab *Geryon quinquedens* and the stone crab *Menippe mercenaria*. *Marine Ecology Progress Series*, **2**, 91–95.
Sulkin, S.D., Blanco, A., Chan, J. & Bryant, M. (1998) The effects of limiting access to prey on the development of the first zoeal stage of the brachyuran crabs *Cancer magister* and *Hemigrapsus oregonensis*. *Marine Biology*, **131**, 515–521.
Summerfelt, S.T. (2003) Ozonation and UV irradiation — an introduction and examples of current applications. *Aquacultural Engineering*, **28**, 21–36.
Summerfelt, S.T. & Hochheimer, J.N. (1997) Review of ozone processes and applications as an oxidizing agent in aquaculture. *The Progressive Fish-Culturist*, **59**, 94–105.
Suprayudi, M.A., Takeuchi, T., Hamasaki, K. & Hirokawa, J. (2002) Effect of *Artemia* feeding schedule and density on the survival and development of larval mud crab *Scylla serrata*. *Fisheries Science*, **68**, 1295–1303.
Suprayudi, M.A., Takeuchi, T. & Hamasaki, K. (2004a) Effects of *Artemia* enriched with eicosapentaenoic and docosahexaenoic acid on survival and occurrence of molting failure in megalop larvae of the mud crab *Scylla serrata*. *Fisheries Science*, **70**, 650–658.
Suprayudi, M.A., Takeuchi, T. & Hamasaki, K. (2004b) Essential fatty acids for larval mud crab *Scylla serrata*: implications of lack of the ability to convert C18 unsaturated fatty acids to highly unsaturated fatty acids. *Aquaculture*, **231**, 403–416.
Suzuki, N., Murakami, K., Takeyama, H. & Chow, S. (2006) Molecular attempt to identify prey organisms of lobster phyllosoma larvae. *Fisheries Science*, **72**, 342–349.
Tacon, A.G.J. (1996) Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems. *Aquaculture Nutrition*, **2**, 165–174.
Takahashi, Y., Itami, T. & Kondo, M. (1995) Immunodefense system of Crustacea. *Fish Pathology*, **30**, 141–150.
Tal, Y., Nussinovitch, A. & van Rijn, J. (2003) Nitrate removal in aquariums by immobilized *Pseudomonas*. *Biotechnology Progress*, **19**, 1019–1021.
Talbot, F.H. & Talbot, M.S. (1971) L'étoile de mer "Couronne d'épines" (*Acanthaster*) et les récifs de la Grande Barrière. *Endeavour*, **30**, 38–42.
Tango, M.S. & Gagnon, G.A. (2003) Impact of ozonation on water quality in marine recirculation systems. *Aquacultural Engineering*, **29**, 125–137.

Teboul, D. (1993) Internal monofilament tags used to identify juvenile *Penaeus monodon*: tag retention and effects on growth and survival. *Aquaculture*, **113**, 167–170.

Teshima, S.-I. (1998) Nutrition of *Penaeus japonicus*. *Reviews in Fisheries Science*, **6**, 97–111.

Teshima, S.-I. & Kanazawa, A. (1971) Biosynthesis of sterols in the lobster, *Panulirus japonica*, the prawn, *Penaeus japonicus*, and the crab, *Portunus trituberculatus*. *Comparative Biochemistry and Physiology*, **38**, 597–602.

Teshima, S.-I. & Kanazawa, A. (1983a) Variation in lipid compositions during the ovarian maturation of the prawn. *Bulletin of the Japanese Society of Scientific Fisheries*, **49**, 957–962.

Teshima, S.-I. & Kanazawa, A. (1983b) Effects of several factors on growth and survival of the prawn larvae reared with microparticulate diets. *Bulletin of the Japanese Society of Scientific Fisheries*, **49**, 1893–1896.

Teshima, S.-I., Kanazawa, A. & Sakamoto, M. (1982) Microparticulate diets for the larvae of aquatic animals. *Mini Review and Data File of Fisheries Research*, **2**, 67–86.

Teshima, S.-I., Kanazawa, A., Koshio, S. & Horinouchi, K. (1988a) Lipid metabolism in destalked prawn *Penaeus japonicus*: induced maturation and accumulation of lipids in the ovaries. *Nippon Suisan Gakkaishi*, **54**, 1115–1122.

Teshima, S., Kanazawa, A., Horinouchi, K. & Koshio, S. (1988b) Lipid metabolism in destalked prawn *Penaeus japonicus*: induced maturation and transfer of lipid reserves to the ovaries. *Nippon Suisan Gakkaishi*, **54**, 1123–1129.

Teshima, S., Koshio, S., Ishikawa, M. & Kanazawa, A. (2001) Protein requirement of the prawn *Marsupenaeus japonicus* estimated by a factorial method. *Hydrobiologia*, **449**, 293–300.

Teshima, S., Koshio, S., Ishikawa, M., Alam, S. & Hernandez Hernandez, L. (2006) Protein requirements of the freshwater prawn *Macrobrachium rosenbergii* evaluated by the factorial method. *Journal of the World Aquaculture Society*, **37**, 145–153.

Thessalou-Legaki, M., Peppa, A. & Zacharaki, M. (1999) Facultative lecithotrophy during larval development of the burrowing shrimp *Callianassa tyrrhena*. *Marine Biology*, **133**, 635–642.

Thessalou-Legaki, M., Zenetos, A., Kambouroglou, V., Corsini-Foka, M., Kouraklis, P., Dounas, C. & Nicolaidou, A. (2006) The establishment of the invasive crab *Percnon gibbesi* (H. Milne Edwards, 1853) (Crustacea: Decapoda: Grapsidae) in Greek waters. *Aquatic Invasions*, **1**, 133–136.

Thiel, M. & Baeza, J.A. (2001) Factors affecting the social behaviour of crustaceans living symbiotically with other marine invertebrates: a modeling approach. *Symbiosis*, **30**, 163–190.

Thiel, M. & Correa, C. (2004) Female rock shrimp *Rhynchocinetes typus* mate in rapid succession up a male dominance hierarchy. *Behavioural Ecology and Sociobiology*, **57**, 62–68.

Thiel, M. & Hinojosa, I. (2003) Mating behavior of female rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea): indication of convenience polyandry and cryptic female choice. *Behavioral Ecology and Sociobiology*, **55**, 113–121.

Thiel, M., Zander, A. & Baeza, J. A. (2003) Movements of the symbiotic crab *Liopetrolisthes mitra* between hosts, black sea urchins *Tetrapygus niger*. *Bulletin of Marine Science*, **72**, 89–101.

Thiesen, D., Stansell, D. & Woods, L. (1998) Disinfection of nauplii of *Artemia franciscana* by ozonation. *The Progressive Fish Culturist*, **60**, 149–151.

Thomas, M., van Vliet, T. & van der Poel, A.F.B. (1998) Physical quality of pelleted animal feed 3. Contribution of feedstuff components. *Animal Feed Science and Technology*, **70**, 59–78.

Thompson, A.R., Thacker, C.E. & Shaw, E.Y. (2005) Phylogeography of marine mutualists: parallel patterns of genetic structure between obligate goby and shrimp partners. *Molecular Ecology*, **14**, 3557–3572.

Thompson, J.N. (1994) *The Coevolutionary Process*. University of Chicago Press, Chicago, Illinois, USA.

Thorson, G. (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, **25**, 1–45.

Thuy, N.T.B. & Ngoc, N.B. (2004) Current status and exploitation of wild spiny lobsters in Vietnamese waters. In: *Proceedings of a Workshop on ACIAR* (ed. K.C. Williams) pp. 13–16. Institute of Oceanography, Nha Trang, Vietnam.

Tidwell, J.H., Coyle, S., Webel, C. & Evans, J. (1999) Effects and interactions of stocking density and added substrate on production and population structure of freshwater prawns *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society*, **30**, 174–179.

Tidwell, J.H., Coyle, S., Van Arnum, A. & Webel, C. (2000) Production response of freshwater prawns *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. *Journal of the World Aquaculture Society*, **31**, 452–458.

Tidwell, J.H., Coyle, S., Van Arnum, A. & Webel, C. (2002) Effects of substrate amount and orientation on production and population structure of freshwater prawns *Macrobrachium rosenbergii* in ponds. *Journal of the World Aquaculture Society*, **33**, 63–69.

Timmons, M.B., Summerfelt, S.T. & Vinci, B.J. (1998) Review of circular tank technology and management. *Aquacultural Engineering*, **18**, 51–69.

Tirmizi, N. & Kazmi, M. (1971) Sexual dimorphism in *Saron marmoratus* (Olivier) (Decapoda, Hippolytidae). *Crustaceana*, **21**, 283–293.

Tlusty, M.F. (2002) The benefits and risks of aquacultural production for the aquarium trade. *Aquaculture*, **205**, 203–219.

Tlusty, M.F. (2004) Ornamental aquaculture — small scale of production does not automatically mean small scale of impact. *OFI Journal*, **46**, 6–9.

Tlusty, M.F., Goldstein, J.S. & Fiore, D.R. (2005a) Hatchery performance of early benthic juvenile American lobsters (*Homarus americanus*) fed enriched frozen adult *Artemia* diets. *Aquaculture Nutrition*, **11**, 191–198.

Tlusty, M.F., Fiore, D.R. & Goldstein, J.S. (2005b) Use of formulated diets as replacements for *Artemia* in the rearing of juvenile American lobsters (*Homarus americanus*). *Aquaculture*, **250**, 781–795.

Tolomei, A., Crear, B. & Johnston, D. (2003) Diet immersion time: effects on growth, survival and feeding behaviour of juvenile southern rock lobster, *Jasus edwardsii*. *Aquaculture*, **219**, 303–316.

Tong, L., Moss, G., Paewai, M. & Pickering, T. (1997) Effect of brine-shrimp numbers on growth and survival of early stage phyllosoma larvae of the rock lobster *Jasus edwardsii*. *Marine and Freshwater Research*, **48**, 935–940.

Tong, L., Moss, G., Pickering, T. & Paewai, P. (2000) Temperature effects on embryo and early larval development of the spiny lobster *Jasus edwardsii*, and description of a method to predict larval hatch times. *Marine and Freshwater Research*, **51**, 243–248.

Toonen, R.J. & Wee, C.B. (2005) An experimental comparison of sediment-based biological filtration designs for recirculating aquarium systems. *Aquaculture*, **250**, 244–255.

Tóth, E. & Duffy, J.E. (2005) Coordinated group response to nest intruders in social shrimp. *Biology Letters*, **1**, 49–52.

Touir, A. (1977) Données nouvelles concernant l'endocrinologie sexuelle des Crustacés Décapodes Natantia hermaphrodites et gonochoriques. Mise en évidence d'un contrôle neurohormonal du maintien de l'áppareil génital mâle et des glandes androgénes exercé par le protocérébron médian. *Comptes Rendus des Séances de la Société de Biologie*, **285**, 539–542.

Trager, G. & Genin, A. (1993) Flow velocity induces a switch from active to passive suspension feeding in a porcelain crab *Petrolisthes leptocheles* (Heller). *Biological Bulletin*, **185**, 20–27.

Treece, G.D. & Fox, J.M. (1993) *Design, Operation and Training Manual for an Intensive Culture Shrimp Hatchery.* Texas A&M University, Sea Grant Collection Program, Galveston, Texas, USA.

Triño, A.T., Millamena, O.M. & Keenan, C. (1999) Commercial evaluation of monosex pond culture of the mud crab *Scylla* species at three stock densities in the Philippines. *Aquaculture*, **174**, 109–118.

Trivers, R.L. (1971) The evolution of reciprocal altruism. *Quarterly Reviews in Biology*, **46**, 35–57.

Tuan, L.A., Nho, N.T. & Hambrey, J. (2000) Status of cage mariculture in Vietnam. In: *Proceedings of the First International Symposium on Cage Aquaculture in Asia* (eds I.C. Liao & C.K. Lin) pp. 111–123. Asian Fisheries Society, Manila, and World Aquaculture Society, Southeast Asian Chapter, Bangkok, Thailand.

Tucker, J.W., Jr (1998) *Marine Fish Culture*. Kluwer Academic Publishers, Boston, USA.

Tunberg, B.G. & Creswell, R.L. (1988) Early growth and mortality of the Caribbean king crab *Mithrax spinosissimus* reared in the laboratory. *Marine Biology*, **98**, 337–343.

Turnbull, T. (1981) A study of the symbiotic relationship between the palaemonid shrimp *Periclimenes pedersoni* Chace (Crustacea, Decapoda, Caridea) and certain species of serranid fishes in the Bahamas. PhD thesis, New York University.

Turra, A. (2003) Shell condition and adequacy of three sympatric intertidal hermit crab populations. *Journal of Natural History*, **37**, 1781–1795.

Udekem d'Acoz, C. d' (2003) *Lysmata seticaudata* (Risso, 1816) and *L. nilita* Dohrn & Holthuis, 1950 are protandrous simultaneous hermaphrodites (Decapoda, Caridea, Hippolytidae). *Crustaceana*, **75**, 1149–1152.

Vadstein, O. (1997) The use of immunonutrition in marine larviculture: possibilities and changes. *Aquaculture*, **155**, 401–417.

van der Meeren, T. & Naas, K.E. (1997) Development of rearing techniques using large enclosed ecosystems in the mass production of marine fish fry. *Reviews in Fisheries Science*, **5**, 367–390.

Van Montfrans, J., Ryer, C.H. & Orth, R.J. (2003) Substrate selection by blue crab *Callinectes sapidus* megalopae and first juvenile instars. *Marine Ecology Progress Series*, **260**, 209–217.

Van Son, T.C. & Thiel, M. (2006) Mating behaviour of male rock shrimp, *Rhynchocinetes typus* (Decapoda: Caridea): effect of recent mating history and predation risk. *Animal Behaviour*, **71**, 61–70.

Van Tassell, J., Brito, A. & Bortone, S. (1994) Cleaning behaviour among marine fishes and invertebrates in the Canary Islands. *Cybium*, **18**, 117–127.

Van Wyk, P.M. (1982) Inhibition of the growth and reproduction of the porcellanid crab *Pachycheles rudis* by the bopyrid isopod, *Aporobopyrus muguensis*. *Parasitology*, **85**, 459–473.

Vance, R.R. (1972) Competition and mechanisms of coexistence in three sympatric species of intertidal hermit crabs. *Ecology*, **53**, 1062–1074.

Vanhaecke, P. & Sorgeloos, P. (1980) International Study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. In: *The Brine Shrimp Artemia*, Vol. 3 (Ecology, Culturing, Use in Aquaculture) (eds G. Persoone, P. Sorgeloos, O. Roels & E. Jaspers) pp. 393–405. Universa Press, Wetteren, Belgium.

Vannini, M. (1985) A shrimp that speaks crab-ese. *Journal of Crustacean Biology*, **5**, 160–167.

Vaseeharan, B. & Ramasamy, P. (2003) Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letters in Applied Microbiology*, **36**, 83–87.

Verity, P.G. & Smetacek, V. (1996) Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Marine Ecology Progress Series*, **130**, 277–293.

Verlaque, M. & Fritayre, P. (1994) Mediterranean algal communities are changing in face of the invasive alga *Caulerpa taxifolia*. *Oceanologica Acta*, **17**, 659–672.

Verschuere, L., Rombout, G., Sorgeloos, P. & Verstraete, W. (1999a) Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, **64**, 655–671.

Verschuere, L., Rombaut, G., Huys, G., Dhont, J., Sorgeloos, P. & Verstraete, W. (1999b) Microbial control of the culture of *Artemia* juveniles through preemptive colonization by selected bacterial strains. *Applied and Environmental Microbiology*, **65**, 2527–2533.

Verschuere, L., Rombaut, G., Sorgeloos, P. & Verstraete, W. (2000a) Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, **64**, 655–671.

Verschuere, L., Heang, H., Criel, G., Sorgeloos, P. & Verstraete, W. (2000b) Selected bacterial strains protect *Artemia* spp. from pathogenic effects of *Vibrio proteolyticus* CW8T2. *Applied and Environmental Microbiology*, **66**, 1139–1146.

Versluis, M., Schmitz, B., von der Heydt, A. & Lohse, D. (2000) How snapping shrimp snap: through cavitating bubbles. *Science*, **289**, 2114–2117.

Vidal, E.A.G., DiMarco, P. & Lee, P. (2006) Effects of starvation and recovery on the survival, growth and RNA/DNA ratio in loliginid squid paralarvae. *Aquaculture*, **260**, 94–105.

Vincent, M., Ramos, L. & Oliva, L. (1988a) Variations qualitatives et quantitatives des pigments caroténoïdes dans l'ovaire et l'hépatopancréas de *Penaeus schmitti* au cours de la maturation ovarienne. *Archives Internationales de Physiologie et de Biochimie*, **96**, 155–164.

Vincent, M., Ramos, L. & Oliva, L. (1988b) Midgut gland as monitor organ for the nutritional value of diets in *Penaeus monodon* (Decapoda). *Aquaculture*, **48**, 1–12.

Virtue, P., Mayzaud, P., Albessard, E. & Nichols, P.D. (2000) Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat, and Mediterranean waters. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 104–114.

Vytopil, E. & Willis, B.L. (2001) Epifaunal community structure in *Acropora* spp. (Scleractinia) on the Great Barrier Reef: implications of coral morphology and habitat complexity. *Coral Reefs*, **20**, 281–288.

Wabnitz, C., Taylor, M., Green, E. & Razak, T. (2003) *From Ocean to Aquarium*. United Nations Environment Programme — World Conservation Monitoring Center, Cambridge, UK.

Wagner, H.P. (1990) The genera *Mithrax* Latreille, 1818 and *Mithraculus* White, 1847 (Crustacea: Brachyura: Majidae) in the Western Atlantic Ocean. *Zooloogische Verhandelingen*, **264**, 1–65.

Wagner, M., Durbin, E. & Buckley, L. (1998) RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. *Marine Ecology Progress Series*, **162**, 173–181.

Wahle, R.A. & Steneck, R.S. (1992) Habitat restrictions in early benthic life: experiments on habitat selection and in situ predation with the American lobster. *Journal of Experimental Marine Biology and Ecology*, **157**, 91–114.

Waloszek, D. & Maas, A. (2005) The evolutionary history of crustacean segmentation: a fossil-based perspective. *Evolution & Development*, **7**, 515–527.

Wasserthal, L.T. & Seibt, U. (1976) Feinstruktur, Function und Reinigung der Antennalen sinneshaare der Garnele *Hymenocera picta*. *Zeitschrift für Tierpsychologie*, **42**, 186–199.

Watanabe, T., Kitajima, C. & Fujita, S. (1983) Nutritional values of live organisms used in Japan for mass propagation of fish: a review. *Aquaculture*, **34**, 115–143.

Watson, I. (2000) *The Role of the Ornamental Fish Industry in Poverty Alleviation*. Natural Resources Institute, Kent, UK, Project No. V0120.

Weber, J.C. & Epifanio, C.E. (1996) Response of mud crab (*Panopeus herbstii*) megalopae to cues from adult habitat. *Marine Biology*, **126**, 655–661.

Wedemeyer, G.A. (1996) *Physiology of Fish in Intensive Culture*. International Thompson Publishing, New York, USA.

Wehrtmann, I.S. & Graeve, M. (1998) Lipid composition and utilization in developing eggs of two tropical marine caridean shrimps (Decapoda: Caridea: Alpheidae: Palaemonidae). *Comparative Biochemistry and Physiology, Part B*, **121**, 457–463.

Wehrtmann, I.S. & Kattner, G. (1998) Changes in volume, biomass, and fatty acids of developing eggs in *Nauticaris magellanica* (Decapoda: Caridea): a latitudinal comparison. *Journal of Crustacean Biology*, **18**, 413–422.

Wehrtmann, I.S. & López, G.A. (2003) Effects of temperature on the embryonic development and hatchling size of *Betaeus emarginatus* (Decapoda: Caridea: Alpheidae). *Journal of Natural History*, **37**, 2165–2178.

Welch, J.M., Rittschof, D., Bullock, T.M. & Forward, R.B., Jr (1997) Effects of chemical cues on settlement behavior of blue crab *Callinectes sapidus* postlarvae. *Marine Ecology Progress Series*, **154**, 143–153.

Weston, D.P. (1996) Environmental considerations in the use of antibacterial drugs in aquaculture. In: *Aquaculture and Water Resource Management* (eds D. Baird, M.V.M. Beveridge, L.A. Kelly & J.F. Muir) pp. 140–165. Blackwell, Oxford, UK.

Wheatly, M.G. (1996) An overview of calcium balance in crustaceans. *Physiological Zoology*, **69**, 351–382.

Wheatly, M.G. (1999) Calcium homeostasis in Crustacea: the evolving role of branchial, renal and hypodermal ephithelia. *Journal of Experimental Zoology*, **283**, 620–640.

Wheatly, M.G., Zanotto, F.P. & Hubbard, M.G. (2002) Calcium homeostasis in crustaceans: subcellular Ca dynamics. *Comparative Biochemistry and Physiology, Part B*, **132**, 163–178.

Whitfield, P., Gardner, T., Vives, S.P., Gilligan, M.R., Courtenay, W.R., Carleton, R. & Hare, J.A. (2002) Biological invasions of the Indo-Pacific lionfish (*Pterois volitans*) along the Atlantic coast of North America. *Marine Ecology Progress Series*, **235**, 289–297.

Wickler, W. (1973) Biology of *Hymenocera picta* Dana. *Micronesia*, **9**, 225–230.

Wickler, W. & Seibt, U. (1970) Das Verhalten von *Hymenocera picta* Dana, einer seesterne fressenden Garnele. *Zeitschrift für Tierpsychologie*, **27**, 352–368.

Wicksten, M.K. (1998) Behaviour of cleaners and their client fishes at Bonaire, Netherlands Antilles. *Journal of Natural History*, **32**, 13–30.

Wilber, D.H. & Wilber, T.P., Jr (1989) The effects of holding space and diet on the growth of the West Indian spider crab *Mithrax spinosissimus* (Lamarck). *Journal of Experimental Marine Biology and Ecology*, **131**, 215–222.

Wilber, D.H. & Wilber, T.P., Jr (1991) Environmental influences on the growth and survival of West Indian spider crabs *Mithrax spinosissimus* (Lamarck) in culture. *Journal of Experimental Marine Biology and Ecology*, **146**, 27–38.

Williams, K.C., Smith, D.M., Irvin, S.J., Barclay, M.C. & Tabrett, S.J. (2005) Water immersion time reduces the preference of juvenile tropical spiny lobster *Panulirus ornatus* for pelleted dry feeds and fresh mussel. *Aquaculture Nutrition*, **11**, 415–426.

Williams, M.J. (1981) Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). *Journal of Experimental Marine Biology and Ecology*, **52**, 103–113.

Williamson, D.I. (1969) Names of larvae in the Decapoda and Euphasiacea. *Crustaceana*, **16**, 210–213.

Williamson, D.I. (1970) On a collection of planktonic Decapoda and Stomatopoda (Crustacea) from the East Coast of Sinai Peninsula, Northern Red Sea. *Bulletin of Sea Fisheries Research Station, Haifa*, **56**, 1–48.

Williamson, D.I. (1976) Larvae of Stenopodidae (Crustacea, Decapoda) from the Indian Ocean. *Journal of Natural History*, **10**, 497–509.

Williamson, D.I. (1982) Larval morphology and diversity. In: *The Biology of Crustacea*, Vol. 2 (Embryology, Morphology and Genetics) (ed. L.G. Abele) pp. 43–110. Academic Press, New York.

Wilson, K.A., Scotto, L.E. & Gore, R.H. (1979) Studies on decapod Crustacea from the Indian River region of Florida XIII. Larval development under laboratory conditions of the spider crab *Mithrax forceps* (A. Milne-Edwards, 1875) (Brachyura: Majidae). *Proceedings of the Biological Society of Washington*, **92**, 307–327.

Wirtz, P. (1995) *Unterwasserführer Madeira Azoren Kanaren Niedere Tiere*. Delius Klasing Verlag, Bielefeld, Germany.

Wirtz, P. (1997) Crustacean symbionts of the sea anemone *Telmatactis cricoides* at Madeira and Canary Islands. *Journal of Zoology, London*, **242**, 799–811.

Wirtz, P. & Debelius, H. (2003) *Mediterranean and Atlantic Invertebrate Guide*. ConchBooks, Hackenheim, Germany.

Wittenrich, M.L. (2002) Breeding *Lysmata rathbunae* shrimp; metamorphosis in 19 days. *SeaScope*, **19**, 1–4.

Wolcott, D.L. & De Vries, M.C. (1994) Offshore megalopae of *Callinectes sapidus*: depth of collection, molt stage, and response to estuarine cues. *Marine Ecology Progress Series*, **109**, 157–163.

Wouters, R., Gomez, L., Lavens, P. & Calderon, J. (1999) Feeding enriched *Artemia* biomass to *Penaeus vannamei* broodstock: its effect on reproductive performance and larval quality. *Journal of Shellfish Research*, **18**, 651–656.

Wouters, R., Lavens, P., Nieto, J. & Sorgeloos, P. (2001) Penaeid shrimp broodstock nutrition: an updated review on research and development. *Aquaculture*, **202**, 1–21.

Wunsch, M. (1996) Larval development of *Lysmata amboinensis* (De Man, 1888) (Decapoda: Hippolytidae) reared in the laboratory with a note on *L. debelius* (Bruce, 1983). MS thesis, University of Wales, Bangor, UK.

Wyban, J., Martinez, G. & Sweeney, J. (1997) Adding paprika to *Penaeus vannamei* maturation diet improves nauplii quality. *World Aquaculture*, **28**, 59–62.

Xu, X.L., Ji, W.J., Castell, J.D. & O'Dor, R.K. (1994) Essential fatty acid requirement of the Chinese prawn *Penaeus chinensis*. *Aquaculture*, **127**, 29–40.

Yaldwyn, J.C. (1966) Notes on the behaviour in captivity of a pair of banded coral shrimps, *Stenopus hispidus* (Olivier). *Australian Zoologist*, **13**, 377–389.

Yanagisawa, Y. (1984) Studies on the interspecific relationship between gobiid fish and snapping shrimp. II. Life history and pair formation of snapping shrimp *Alpheus bellulus*. *Publications of the Seto Marine Biological Laboratory*, **29**, 93–116.

Yang, H.J. & Okuno, J. (2004) First larvae of *Lebbeus comanthi* and *Thor amboinensis* (Decapoda: Hippolytidae) in the laboratory. *Korean Journal of Biological Sciences*, **8**, 19–25.

Yang, W.T. (1976) Studies on the western Atlantic arrow crab genus *Stenorhynchus* (Decapoda, Brachyura, Majidae). I. Larval characters of two species and comparison with other larvae of Inachinae. *Crustaceana*, **31**, 157–177.

Yasharian, D., Coyle, S.D., Tidwell, J.H. & Stilwell, W.E. (2005) The effect of tank colouration on survival, metamorphosis rate, growth and time to metamorphosis freshwater prawn (*Macrobrachium rosenbergii*) rearing. *Aquaculture Research*, **36**, 278–283.

Yokes, B. & Galil, B.S. (2006) Touchdown — first record of *Percnon gibbesi* (H. Milne Edwards, 1853) (Crustacea: Decapoda: Grapsidae) from the Levantine coast. *Aquatic Invasions*, **1**, 130–132.

Yoshimura, K.A., Hagiwara, A., Yoshimatsu, T. & Kitajima, C. (1996) Culture technology of marine rotifers and implication for intensive culture of marine fish in Japan. *Marine and Freshwater Research*, **47**, 217–222.

Yoshino, K. & Goshima, S. (2002) Sexual dominance in hermit crab shell fights: asymmetries in owner–intruder status, crab size, and resource value between sexes. *Journal of Ethology*, **20**, 63–69.

Yúfera, M., Rodriguez, A. & Lubián, L.M. (1984) Zooplankton ingestion and feeding behavior of *Penaeus kerathurus* larvae reared in the laboratory. *Aquaculture*, **42**, 217–224.

Zandee, D.I. (1967) Absence of cholesterol synthesis as contrasted with the presence of fatty acid synthesis in some arthropods. *Comparative Biochemistry and Physiology*, **20**, 811–822.

Zandee, D.I. & Kruitwagen, E.C. (1975) Depot sterols in comparisons with structural sterols in *Cancer pagurus* and *Eriocheir sinensis*. *Netherlands Journal of Sea Research*, **9**, 214–221.

Zhang, D., Lin, J. & Creswell, R.L. (1997a) Effect of eyestalk ablation on molt cycle and reproduction in the banded coral shrimp, *Stenopus hispidus* (Oliver). *Journal of Shellfish Research*, **16**, 363–366.

Zhang, D., Lin, J. & Creswell, R.L. (1997b) Larviculture and effect of food on larval survival and development in golden coral shrimp *Stenopus scutellatus*. *Journal of Shellfish Research*, **16**, 367–369.

Zhang, D., Lin, J. & Creswell, R.L. (1998a) Mating behavior and spawning of the banded coral shrimp *Stenopus hispidus* in the laboratory. *Journal of Crustacean Biology*, **18**, 511–518.

Zhang, D., Lin, J. & Creswell, R.L. (1998b) Ingestion rate and feeding behaviour of the peppermint shrimp *Lysmata wurdemanni* on *Artemia* nauplii. *Journal of the World Aquaculture Society*, **29**, 97–103.

Zhang, D., Lin, J. & Creswell, R.L. (1998c) Effects of food and temperature on survival and development in the peppermint shrimp *Lysmata wurdemanni*. *Journal of the World Aquaculture Society*, **29**, 471–476.

Zhang, D. & Lin, J. (2004) Fertilization success without anterior pleopods in *Lysmata wurdemanni* (Decapoda: Caridea), a protandric simultaneous hermaphrodite. *Journal of Crustacean Biology*, **24**, 470–473.

Zhang, D. & Lin, J. (2005a) Development of sexual morphs in two simultaneous hermaphroditic shrimp, *Lysmata boggessi* and *L. wurdemanni*. *Invertebrate Reproduction and Development*, **47**, 11–17.

Zhang, D. & Lin, J. (2005b) Mating without anterior pleopods in a simultaneous hermaphroditic shrimp, *Lysmata wurdemanni* (Decapoda, Caridea). *Crustaceana*, **77**, 1203–1212.

Zhang, D. & Lin, J. (2005c) Comparative mating success of smaller male-phase and larger male-role euhermaphrodite-phase shrimp, *Lysmata wurdemanni* (Caridea: Hippolytidae). *Journal of the Marine Biological Association of the United Kingdom*, **85**, 101–106.

Zhang, D. & Lin, J. (2006) Mate recognition in a simultaneous hermaphroditic shrimp, *Lysmata wurdemanni* (Caridea: Hippolytidae). *Animal Behaviour*, **71**, 1191–1196.

Zhang, D., Rhyne, A.L. & Lin, J. (2007) Density-dependent effect on reproductive behaviour of *Lysmata amboinensis* and *L. boggessi* (Decapoda: Caridea: Hippolytidae). *Journal of the Marine Biological Association of the United Kingdom*, **87**, 517–522.

Ziegler, T.A. & Forward, R.B., Jr (2006) Larval release behaviors of the striped hermit crab, *Clibanarius vittatus* (Bosc): temporal pattern in hatching. *Journal of Experimental Marine Biology and Ecology*, **335**, 245–255.

Zion, B., Shklyar, A. & Karplus, I. (2000) In-vivo fish sorting by computer vision. *Aquacultural Engineering*, **22**, 165–179.

Zmora, O. & Shpigel, M. (2006) Intensive mass production of *Artemia* in a recirculated system. *Aquaculture*, **255**, 488–494.

Zmora, O., Avital, E. & Gordin, H. (2002) Results of an attempt for mass production of *Artemia* in extensive ponds. *Aquaculture*, **213**, 395–400.

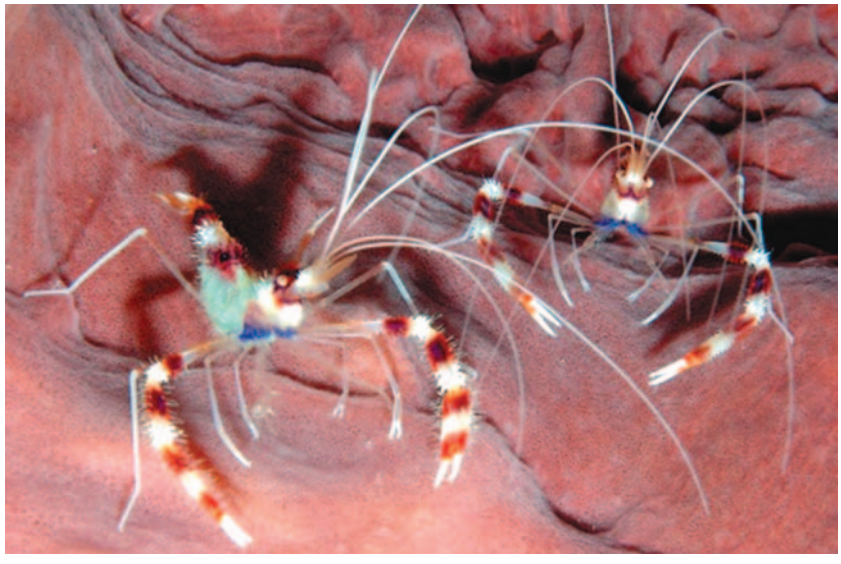
Zmora, O., Findiesen, A., Stubblefield, J., Frenkel, V. & Zohar, Y. (2005) Large-scale juvenile production of the blue crab *Callinectes sapidus*. *Aquaculture*, **244**, 129–139.

Zupo, V. (2000) Effect of microalgal food on sex reversal of *Hippolyte inermis* (Crustacea, Decapoda). *Marine Ecology Progress Series*, **201**, 251–259.

Zupo, V. (2001) Influence of diet on sex differentiation of *Hippolyte inermis* Leach (Decapoda: Natantia) in the field. *Hydrobiologia*, **449**, 131–140.

Zupo, V. & Messina, P. (2007) How do dietary diatoms cause the sex reversal of the shrimp *Hippolyte inermis* Leach (Crustacea, Decapoda). *Marine Biology*, **151**, 907–917.

Index



Color Plate 1 The barber pole or coral banded boxing shrimp *Stenopus hispidus* (photograph by the author).

Color Plate 2 The Mediterranean boxer shrimp *Stenopus spinosus* (Risso, 1827) (photograph by Rui Rosa).

Color Plate 3 The skunk cleaner shrimp *Lysmata amboinensis* carrying embryos under its abdomen (photograph by Ricardo Santos).

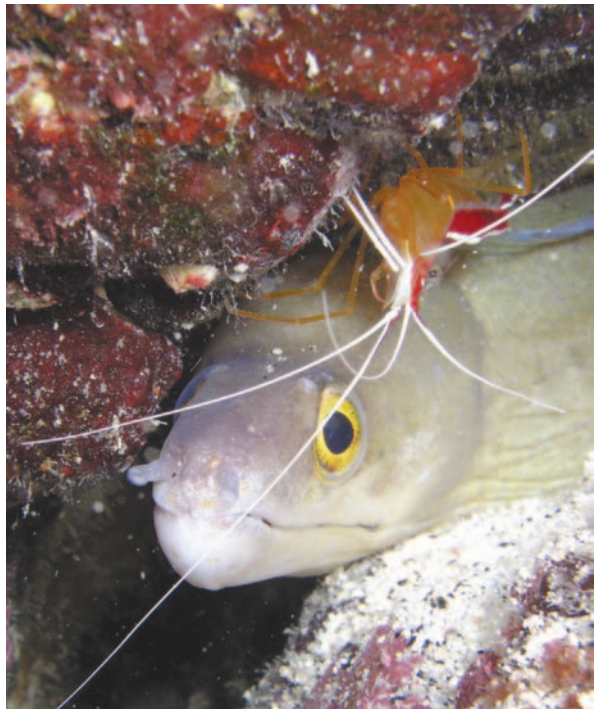
Color Plate 4 The fire shrimp *Lysmata debelius* (photograph by Tiago Garcia).

Color Plate 5 A pair of harlequin shrimp *Hymenocera elegans* eating a starfish (photograph by António Marques).

Color Plate 6 Maturation system for marine ornamental decapods at Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa (photograph by the author).

Color Plate 7 Larval culture system for marine ornamental decapods at Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa using small-scale research tanks (10 liters) (photograph by the author).

Color Plate 8 Newly settled *Lysmata amboinensis*: (A) megalopa and (B) first juvenile (photographs by Luis Magnasco).



Color Plate 9 *Lysmata grabhami* associating with a conger eel in the wild, one of the many fish 'clients' of this cleaner shrimp (photograph by Rui Rosa).

Color Plate 10 Monaco shrimp *Lysmata seticaudata* (Risso, 1816) bred in captivity and only 30 days post-settlement is already displaying the ovarian portion of the gonad producing oocytes (green region in the carapace) (photograph by António Marques).