

Advances in Breeding and Rearing Marine Ornamentals

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Abstract

This work addresses the most relevant advances in the breeding and rearing of marine ornamental species. The main breakthroughs in marine ornamental fish culture are discussed, with seahorses deserving a section of their own as a result of their conservation status and unique biology. Details on spawning, embryo development, larval rearing, plankton culturing, and tank design are presented. In addition, with the increase in popularity of ornamental invertebrates in reef aquariums, details on the culturing techniques of some of the most traded invertebrate groups (e.g., live rocks, corals, anemones, polychaetes, mollusks, decapod crustaceans and echinoderms) are also discussed. Finally, the last part of this work highlights the concerns toward the establishment of sustainable collection, production, and trading practices for marine ornamentals as well as the urgent need to develop reliable traceability protocols to distinguish sustainably caught and/or cultured specimens from wild ones. This work represents not only an exhaustive and updated bibliographical source but also a starting point for all those who want to contribute to the development of this fascinating research field.

Although coral reefs cover less than 1% of the marine environment, they are unanimously considered among the most biologically rich and productive ecosystems on the

Earth. They support over 4000 fish species, about 800 species of reef-building corals and several thousands of other reef invertebrates (cnidarians, sponges, mollusks, crustaceans, and echinoderms; Paulay 1997). The past few decades have been characterized by negative

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anthropogenic effects on coral reef ecosystems, such as sedimentation, nutrient enrichment due to human waste and agriculture run-off, over-fishing, and global climate change (Baskett et al. 2010; Selig and Bruno 2010). The intensive fishing effort required to supply the marine aquarium trade may also have played an important role in the decline of coral reefs. Unlike freshwater ornamental species, where over 90% of fish species are currently produced in captivity, the vast majority of marine aquaria are stocked from wild-caught specimens (Wabnitz et al. 2003). In addition, less conscientious traders continue to support the use of destructive fishing techniques, namely the use of cyanide, to anesthetize highly priced fish species. The use of this poison is known to harm targeted, as well as non-targeted, reef fishes and its deleterious effects on several marine invertebrates are also documented (Barber and Pratt 1998; Hanawa et al. 1998; Mak et al. 2005). The promotion of bleaching in reef-building corals after exposure to cyanide is certainly one of the most dramatic effects of the use of this poison to collect live reef fishes (Jones et al. 1999; Cervino et al. 2003). Nonetheless, it is relevant to highlight that cyanide poisoning is also used to supply the live food fish trade in Southeast Asia (Barber et al. 1997; Pomeroy et al. 2008) and that dynamite fishing is only used to collect reef fishes for human consumption (Pet-Soede et al. 1999).

It is estimated that from oceans to aquaria, up to 80% of the traded animals die during capture, shipment, handling due to the use of poisons during collection, poor handling practices, and diseases (Sadovy and Vincent 2002; Wabnitz et al. 2003). The poor survival of collected specimens through the chain of custody, along with the current dependence of the marine aquarium industry on the collection of wild specimens to supply an ever growing demand, urges researchers to find solutions to make the trade of marine ornamental species a more sustainable practice. Aquaculture is commonly considered a potential alternative, as the captive production of some of the most heavily collected species would certainly contribute to relieving the current fishing pressure on coral

reefs (Tlusty 2002; Pomeroy et al. 2006). This approach may not only generate an alternate supply of marine ornamental specimens but will also allow researchers to collect valuable information about their life history (age at maturity, fecundity, etc.) to improve the management of natural stocks and our understanding of how these organisms respond to human impacts.

This work addresses the most relevant advances in the breeding and rearing of marine ornamental species. The main breakthroughs in marine ornamental fish culture are discussed, with seahorses deserving a section of their own as a result of their conservation status and unique biology. Advances concerning the culture of marine ornamental invertebrates (i.e., live rock production, coral propagation, anemones, polychaetes, mollusks, decapod crustaceans, and echinoderms) are also presented and discussed. The last part of this work highlights the need to establish sustainable collection, production, and trading practices for marine ornamental species as well as to develop reliable traceability protocols to distinguish sustainably caught and/or culture specimens.

Marine Ornamental Fishes (Except Seahorses)

In recent years, there has been an increased focus on supplying aquarium fishes through closed system culturing. The development of reliable and sustainable hatchery procedures for the captive breeding of reef fishes is now becoming essential to reduce pressure on wild populations and also because rearing fish in closed systems is likely to lead to the production of hardier specimens that are far better in captivity and survive longer (Wittenrich 2007). It is hoped that much of the market demand for the more popular ornamentals may eventually be satisfied by cultured fish; however, in reality, most marine ornamental aquaculture remains problematic. In fact, there are numerous critical processes in early life history where deficiencies could represent a limiting factor in captive rearing. Some of the main critical steps are spawning (which includes sexing the fish and the development of a reproductive

competence), embryo development (which is strictly related to broodstock nutrition, maintenance, and genetics), hatching (which depends on the reproductive strategy), and the transition from endogenous to an exogenous feeding by the larvae.

It is well established that the life cycle of most coral reef fishes can be subdivided into three distinct biological/ecological phases: larvae, juvenile, and adult. To cultivate marine animals, we must work on all life stages from eggs to larvae, juveniles, and adults (Holt 2003). In general, the hypothesis of many scientists is that marine ornamental fish can be spawned and raised in captivity and second, the culture techniques developed in the research laboratories can be transferred to commercial production (Holt 2003; Olivotto et al. 2008a).

To start, breeding and cultivating marine ornamental broodstock has to be carefully chosen because high-quality breeders are essential for successful larval rearing. When possible, captive-bred specimens should be preferred over wild ones because they are hardier, far better in captivity, and survive longer, as they are young fish (6–12 mo), and do not undergo shipment stress.

As reproductive strategies used by fish are extremely diverse, knowledge of the life history of the species under study is crucial to the success of captive propagation. Development of reproductive competence relies on the integration of a wide variety of internal and external cues. These signals provide critical information on when an animal should reproduce: whether it is of sufficient size or energy status to reproduce (metabolic cues), whether conditions are optimal for reproductive success (environmental cues), and whether an appropriate mate is present (social cues). When attempting to breed a particular species of fish in captivity, it is important to be able to sex the organisms: some fish are of a predetermined sex (gonochoric fish) and are not capable of sex reversal. Couples are usually formed through trial and error or by looking for sexual dimorphism. For example, in both yellow tail damsel, *Chrysiptera parasema*, and striped blenny, *Meiacanthus grammistes*, males

are much more aggressive, whereas females present a rounder vent.

Some fish are able to perform sex reversal (hermaphrodites). There are simultaneous hermaphrodites, where one individual possesses both female and male reproductive tissue and can act as either sex during a single spawning event. Physical adaptation usually prevents self-fertilization. They represent a minority among aquarium fishes (e.g., Serranidae). Sequential hermaphrodites are dominant and involve an individual acting as one sex during the early part of its life and eventually, if conditions are appropriate, changing sex. They can be subdivided in protogynous (sex change in the female–male direction) and protrandrous (sex change in the male–female direction). Typical examples of protrandrous species are clownfishes where the social status determines the sex of the fish. The female is the largest, the male is the second largest, and the nonbreeders are progressively smaller as the hierarchy descends. Typical protogynous species are dottybacks and angelfishes. After determining the sex of the fish, the hypothalamus, pituitary gonadal axis should be activated. Environmental conditions, including photoperiod, temperature, and food availability may be very important for this activation. Photoperiod and temperature may be manipulated using light timers and heat pumps. Controlled environments mimic natural spawning conditions with seasonal changes in day length and temperature to promote spawning. Usually, for demersal spawners, high temperature (28 C) and long photoperiod (14 L/10 D) are sufficient to get fishes to spawn (Olivotto et al. 2003; Olivotto and Carnevali 2004; Avella et al. 2007), although other combinations (13 L/11 D and 12 L/12 D at 26 C for *Elacatinus figaro*) may work as well (Meirelles et al. 2009; Shei et al. 2010). However, for pelagic spawners, seasonal changes are needed (Holt and Riley 2001) to induce spawning, with fish being subjected to winter (22 C 10 L/14 D), spring (24 C 12 L/12 D), and summer (27–28 C 14 L/10 D) temperatures/photoperiods (Holt and Riley 2001). Nonetheless, spawning always occurs during the summer period (Holt and Riley 2001; Olivotto et al. 2006a).

These temperature and photoperiod conditions, together with good water quality (ensured by suitable filtration equipment) and with heavy feeding, generally result in the spawning of marine ornamental fish in captivity.

The two dominant modes of egg release among marine fish are demersal and pelagic spawning. Demersal spawners usually produce eggs that are attached to a solid surface or spawned in small caves as gelatinous egg masses in the case of egg ball layers. Demersal spawning requires parental care. Typically, the male takes care of the fry until they hatch. In the meantime, the female is involved in feeding to sustain oogenesis. The effort put into parental care depends on the water circulation in the tank; the more the current, the less frequently the male has to fan the embryos. Males have often been observed mouthing the embryos. This is a common practice that may be important in displacing improperly developing embryos.

Pelagic spawners display complex courtship with eggs and sperm being released into the water column. Eggs are usually smaller and produced in greater number when compared with those of demersal spawners. Spawning usually occurs at dusk, as during this transition period, the predatory pressure is reduced at the reef (diurnal predators are seeking refuge and nocturnal ones are still not fully active). Depending on the reproductive strategy, embryo development lasts from hours in pelagic spawners (Brothers and Thresher 1985; Wellington and Victor 1989; Bonhomme and Planes 2000) to days in demersal ones (Russell 1971; Alcalá and Cabanban 1986; Moe 1992, 1997; Brons 1996; Allen 2000). Although the embryo is developing protected by the chorionic membrane, several different biological processes are initiated to prepare it for its independent life in the environment. After a species-specific process of development, the embryo changes into a form that obtains nutrients from external sources, a stage that is achieved through the breakdown of the chorionic membrane (hatching) in demersal species. Hatching has an enzymatic and a mechanical aspect caused by the synchronized action of embryonic movements,

hatching enzymes, and, in demersal spawners and egg-ball layers, parental care (Inhoaya et al. 1997; Olivotto et al. 2004). In several species, successful hatching may depend on ambient light conditions, with most of demersal spawners hatching at night. The development of this photo-regulated hatching is most probably an ecologically meaningful life strategy to reduce the predatory pressure on these larvae.

At hatching, the delicate larvae and prolarvae are extremely sensitive to any turbulence and chemical–physical environmental variation (Brons 1995; Holt 2003; Olivotto et al. 2003). Demersal-spawned larvae develop in the egg until pigmented eyes and a finfold have developed. Larvae are competent at this stage with functional jaws and pigment in the eyes, the yolk sac is almost exhausted and mouth and digestive tract are open and functional. However, pelagic-spawned larvae are very tiny, hatch as prolarvae, and lack pigmented eyes, a digestive system, or mouth. At this stage, they still have large yolk reserves which are used to undergo a second developmental phase of about 48 h in the water column. After this period, the prolarvae have developed into active larvae with pigmented eyes and functional digestive system. Prolarvae are extremely vulnerable to predation during this early period.

As larvae and prolarvae are very delicate during this early part of their life history, different larval rearing systems have been developed to mimic the open ocean's conditions where food organisms are abundant and predators are few. Microcosms made of 20-L glass tanks have been successfully used for several demersal spawners including clownfishes (*Amphiprion* spp.), gobies (*Gobiosoma* spp.), dottybacks (*Pseudochromis* spp.), and some blennies (*Meiacanthus* spp.; Olivotto et al. 2005, 2009). The sides of the tank are covered with black panels to reduce light reflection, whereas the bottom is usually white to facilitate bottom cleaning. The water in these tanks is changed up to 10 times a day through a dripping system (Olivotto et al. 2003; Olivotto and Carnevali 2004). For prolarvae, small 50-L mesh baskets placed in large tanks (400–1000 L) have been used with success.

The use of these rearing chambers concentrates larvae and food, increases food encounter rates, provides shelter, and decreases potential physical damage. Moreover, heating and aeration are carried out in the large tank and the water in the rearing chamber is very clean, warm, and the salinity matches that of the open ocean – all conditions that larvae might naturally encounter (Holt 2003).

The main obstacle in ornamental fish larviculture is the transition from endogenous to exogenous feeding by the larvae and because the larval cycle is spent in the open ocean, this particular environment should be mimicked. The open ocean in the tropics is characterized by warm, calm waters and appropriate concentration of live prey on which larvae are able to feed. From several field studies, it is evident that in the wild, marine fish larvae mainly feed on wild plankton composed of copepods, protozoan, and larvae of benthic organisms. Recent studies have shown that after feeding marine fish larvae with wild plankton and checking their gut contents, the most abundant ingested live prey were copepod eggs and nauplii (Olivotto et al. 2006a; Baensch and Tamaru 2009a, 2009b). Unfortunately, copepods are difficult to culture on a continuous basis and most of the marine fish species are reared using rotifers, *Brachionus* spp., and *Artemia* spp. nauplii. Despite apparent practical advantages in production, rotifers and *Artemia* are not the best live prey for marine fish larvae, as they are not their natural food. For some tiny larvae (butterfly fish, angelfishes, and groupers), these prey are too large, their locomotory patterns (slow circular movement) do not promote predatory activity, and they do not display a fatty acid profile that matches the nutritional requirements of marine fish larvae. Our recent studies (Olivotto et al. 2006b) demonstrated the importance of food enrichment of rotifers and *Artemia* for sunrise dottyback, *Pseudochromis flavivertex*, larvae. Larvae were divided into experimental groups fed on different enriched and unenriched live prey. Larvae fed on nonenriched rotifers did not survive past day 7, whereas highest survival rates (39% juveniles) were observed in larvae fed exclusively on enriched rotifers

and *Artemia*. Moreover, larvae fed enriched live prey showed a faster growth and completed metamorphosis earlier than those fed on nonenriched *Artemia* nauplii. These results clearly indicated that live prey enrichment is essential for rearing this species. In addition, it is well established that anomalous pigmentation, consisting of partial or total lack of white bands (“miss-band”) is a common problem in the production process of the false percula clownfish, *Amphiprion ocellaris* (Avella et al. 2007). As “miss-band” clownfishes are sold at a lower price by the companies, there is a great interest in understanding and solving this problem. It has been demonstrated that highly unsaturated fatty acids (HUFAs) administration in the live prey not only positively affected growth but also reduced the percentage of miss-band organisms (Avella et al. 2007). Poor color performance of juveniles and adults can also reduce their price, although this can be easily manipulated through either diet supplements rich in carotenoids (Ho et al. 2008) or light intensity and background (Yasir and Kim 2009a, 2009b).

Thus, there is a strong need for identification of alternative food sources that do not have the inadequacies of rotifers and *Artemia* and that can increase the variety, growth, and survival of the species that can be cultivated. Adult copepods, as well as copepodites and nauplii, are the food items preferred by fish larvae in the wild and when used as live prey (solely or in combination with rotifers and *Artemia* nauplii), they usually dominate the gut content of larvae (Holt 2003). Delbare et al. (1996) summarized the advantages of using copepods in larviculture, such as the wide range of body size between nauplii and adults, their typical movement, and their high content of HUFAs. These fatty acids, in particular eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are extremely important for larval fish survival and growth and several studies have demonstrated that they are essential in larval diets (Sargent et al. 1989). Deficiencies in these fatty acids can cause a general decrease in larval health, poor growth, low feed efficiency, anemia, and high mortality (Sargent

et al. 1999; Bell et al. 2003; Olivotto et al. 2003, 2006b; Faulk and Holt 2005).

With the increasing worldwide interest in aquaculture, copepods may be considered a valid and alternative food source for the culture of many larval fish. The use of cultured copepods in intensive fish larviculture (Van der Meeren and Naas 1997; Papandroulakis et al. 2005) has involved calanoids such as *Acartia* spp. (Schipp et al. 1999), *Eurytemora* spp. (Shields et al. 1999), *Parvocalanus* spp. (Olivotto et al. 2006a), *Centropages typicus* (Olivotto et al. 2008a, 2008b, 2008c, 2009), and harpacticoid copepods such as *Euterpina acutifrons* (Kraul et al. 1992), *Tisbe* spp. (Stottrup and Norsker 1997; Olivotto et al. 2008a, 2008b, 2008c), and *Trigriopus japonicus* (Fukusho 1980). Harpacticoids are easier to culture at higher densities but are predominantly found on the tank walls rather than in the water column. For this reason, these live preys are less available to fish larvae and are commonly used as a supplement to the traditional rotifers/*Artemia* diet (Olivotto et al. 2008a, 2008b).

The best results in larviculture have been obtained using calanoid copepods which have a high content of HUFAs, are entirely pelagic, and thus more available as prey for marine fish larvae. Usually these copepods have very small naupliar stages, which are more readily captured by fish larvae with small mouth gapes at first feeding (Payne and Rippingale 2001; Olivotto et al. 2006a, 2006b). Unfortunately, there are several difficulties in culturing calanoid copepods on a continuous basis, because they are usually cultured at very low densities, in large tanks, and need to be fed different algal combinations (Holt 2003). Recently, at the Stazione Zoologica Anton Dohrn in Naples, *C. typicus* (Kröyer, 1849) copepods were cultured through different generations in a 500-L recirculating system (INNOVAQUA srl, Reggio Emilia, Italy) equipped with biological/mechanical filtration. Copepods were fed with a mixture of phytoplanktonic cells of *Heterocapsa niei*, *Tetraselmis suecica*, and *Isochrysis galbana* at concentrations of 5.5×10^3 , 1.25×10^4 , and 3.4×10^4 cells/mL, respectively, corresponding to about 1 mg

cells/L of each algae. Nauplii and copepodites were collected at different developmental stages from naupliar stage I (NI; 110- μ m length) to copepodids III (CIII; 560- μ m length) and used for feeding studies. Nauplii and copepodites were attracted into a 155- to 300- μ m mesh net size filter cage using a light, automatically collected in a 200-L tank, and finally concentrated in 15 L of seawater. This system was able to produce enough nauplii for feeding trials and may represent a starting point for the development of a mass cultivation system for calanoid copepods.

Copepods are in fact the ideal diet for marine fish with very tiny larvae. Good candidates for feeding copepods are angelfishes, Pomacanthidae, which are among the most requested marine species by the ornamental fish market (Baensch and Tamaru 2009a, 2009b). The main problem in culturing these species occurs at first feeding. The tiny larvae are too small to eat rotifers and alternative live preys have to be selected to feed the larvae (Holt 2000, 2003). In a study on lemonpeel angelfish, *Centropyge flavissimus*, larvae fed the circumtropical copepod *Parvocalanus* sp. or wild plankton (25–75 μ m in size) at 28 C had a 10% survival rate to day 14 (Olivotto et al. 2006a). In addition, a similar experiment was performed on semi-circle angelfish, *Pomacanthus semicirculatus*. Different diets were tested on larval survival rate and a diet composed of 30% *Gonyaulax* sp. (dinoflagellate) + 35% *Nannochloropsis* sp. + 35% *Brachionus rotundiformis* was the best choice for larval *P. semicirculatus*. Further studies are necessary to close the reproductive cycle of this species (Leu et al. 2009). A small-scale culture-technology for *Parvocalanus* spp. copepods was used to successfully rear some “difficult species” such as the flame angel fish, *Centropyge loriculus*, and the yellow tang, *Zebrasoma flavescens* (Laidley et al. 2009). At present, scientists still need to increase larval survival through late larval period when it becomes increasingly difficult to maintain sufficient numbers of larger copepodites prior to weaning the larvae onto newly hatched *Artemia* nauplii. It has been demonstrated that

two genera of copepods, *Parvocalanus* sp. and *Pseudodiaptomus* sp., offer substantial advantages in culture techniques (in terms of production numbers, size, and survival) compared with the most widely cultured species *Acartia tonsa* (Rhyne et al. 2009a, 2009b).

In conclusion, many fish can be spawned in captivity; the main critical bottleneck is the first feeding. Using rotifers and *Artemia* during the early life history of fish does not always promote optimal larval growth because these live prey may contain an inadequate fatty acid profile and, in some cases, display an inappropriate size (Kahan 1981; Holt 2003; Olivotto et al. 2003; Faulk and Holt 2005). Because of this, there is a need for identification of alternative food sources that do not have these inadequacies and can promote adequate growth (Sun and Fleeger 1995). Adult copepods, along with copepodites and nauplii, are considered good candidates for feeding marine ornamental fish larvae (Holt 2003). The advantages of using copepods in larviculture are mainly related to their wide range of body sizes between nauplii and adults, their typical movement, and their high content of HUFAs. Bottlenecks still remain; the development of a copepod-based commercial production of marine fish still requires the use of large mesocosms. Research should be focused on finding copepod species with short generation times and tolerance to high densities, in addition to gaining a better understanding of the possible involvement of amino acids, protein, pigment, and vitamin contents of copepods in larval fish growth and survival.

Seahorses

A very special group of marine ornamental fishes are the Syngnathidae, a family that includes seahorses, pipefishes, and sea dragons. Seahorses, *Hippocampus* spp., are bony fishes whose evolutionary history is so recent that the major stages of morphological evolution are still represented in extant species (Teske and Behegaray 2009). These iconic fishes are distributed in coastal tropical, subtropical, and temperate marine regions throughout the world

(Kuitert 2000), being very popular in Chinese traditional medicine and the marine ornamental trades (Lourie et al. 1999). Unfortunately, wild populations are declining due to overexploitation (Lourie et al. 1999) and all seahorse species have been listed in the Appendix II of Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES 2002a).

Seahorses are interesting for economical, cultural, scientific, and educational reasons. Because of the increasing demand for seahorses for the aquarium trade and the pressure on wild populations, interest in the biology and rearing of seahorses has increased in recent years. At least 13 species are commercially produced (Koldewey and Martin-Smith 2010), but most seahorse species are potential candidates for the ornamental fish trade. Compared with other reef fishes, rearing of seahorses is a relatively new industry with high economic potential due to increasing demand and high market prices for commercialized species. Breeding of large numbers of quality seahorses for fish trade or the traditional Chinese market (medicine and gastronomy) can also contribute to reduce the pressures on wild seahorse populations.

Ecological, biological, and physiological aspects of seahorses have been almost unknown until recently. There are several characteristics that are unique among reef fishes: low density, limited home ranges, reduced mobility, short life span, parental care, sexual dimorphism, low fecundity, pair bonding, mating with courtship displays, batch spawners with repeated mates within a breeding season, and genetic monogamous pattern within a single breeding season (Foster and Vincent 2004). Although rearing technology exists for a few species at a commercial scale, improvements are necessary to enhance profitability. Some years ago, research efforts were directed toward the development of rearing technology for some tropical or subtropical species (Koldewey and Martin-Smith 2010). More recently, attention has also been focused on temperate species such as the European long-snouted seahorse, *Hippocampus guttulatus* or the European short-snouted seahorse, *H. hippocampus* (Molina et al. 2007;

Otero et al. 2007, 2009, 2010; Faleiro et al. 2008; Palma et al. 2008; Planas et al. 2008a, 2008b, 2009a, 2009b, 2009c; Planas and Quintas 2009). Knowledge on essential aspects is almost lacking, especially on those related with reproductive success and mortality of juveniles. The rearing of seahorses in captivity has contributed to increased understanding of seahorse biology and physiology, but there is still insufficient information on growth, breeding, and feeding/nutritional requirements.

Reproduction in captivity has been successfully achieved in a number of seahorse species but still remains a bottleneck for many others. Knowledge of reproductive physiology, female maturation, or egg characteristics is extremely limited in seahorses (Selman et al. 1991; Poortenaar et al. 2004). In general, mating in captive conditions is not a constraint but seems to be especially difficult in the European long-snouted seahorse, *H. guttulatus* (Faleiro et al. 2008; Planas et al. 2008a). In this species, courtship displays are accompanied by a low breeding success (Planas et al. 2009a). A possible explanation, which needs to be confirmed, could rely on unfulfilled nutritional requirements. Adult seahorses are rarely fed on *Artemia* alone (Ortega-Salas and Reyes-Bustamante 2006; Planas et al. 2008a). Other prey (mysidaceans, amphipods, and shrimps) are usually offered, alone (alive or frozen) or as *Artemia* supplementation (Woods and Valentino 2003; Koldewey 2005; Lin et al. 2006, 2007, 2008b; Ortega-Salas and Reyes-Bustamante 2006; Olivotto et al. 2008a, 2008b, 2008c; Murugan et al. 2009). In captivity, adult seahorses show preference for mysids and amphipods (Vite et al. 2009). In fact, these are their preferred prey in the wild as well (Teixeira and Musick 2001; Kitsos et al. 2008). Adult *Artemia* promotes high growth rates in seahorses (Planas et al. 2009c), but its nutritional adequacy, in terms of reproductive efficiency, has been often questioned. Otero et al. (2009) carried out a comparative study in *H. hippocampus* fed on enriched adult *Artemia* or on wild-collected mysids, concluding that the latter enhanced spawning and quality of newly hatched young. Conversely, female maturation,

clutch size, and brood size were not affected by mysids and improved in cultured *H. guttulatus* fed on enriched *Artemia* (Planas et al. 2009a, 2010), when compared with wild seahorses (Curtis 2007). These findings suggest that receptivity for mating could be partially inhibited by other unknown factors, not necessarily related to feeding/nutritional conditions.

Egg size has been used as a criterion of egg/juveniles quality. Although interspecific differences in egg size within seahorse species depend on latitude rather than female size (Foster and Vincent 2004), intraspecific egg size comparisons would be useful in egg quality studies. In seahorses, eggs are typically asymmetrical, nonbuoyant, and larger (0.9–3.8 mm in diameter) than in most tropical marine fishes (Ahlstrom and Moser 1980). However, because eggs are asymmetrical, measuring them accurately has been problematic (Foster and Vincent 2004) due to the lack of a standardized protocol. A mathematical model has been proposed for *H. guttulatus* egg and yolk size estimation based on length and width measurements (Planas and Quintas 2009). The model could be easily applied to other seahorse species. Egg size has been positively correlated with female size in some pipefish species (Berglund et al. 1986) but not in seahorses (Foster and Vincent 2004; Planas et al. 2010). However, a correlation between clutch size and female size/weight has been found for *H. whitei*, *H. erectus*, and *H. guttulatus* (Teixeira and Musick 2001; Vincent and Giles 2003; Planas et al. 2010). In addition, Curtis (2004) pointed out that male size is a reasonable predictor of brood size in *H. guttulatus*. Accordingly, reproductive efficiency would be enhanced in larger fishes.

Very little is known on feeding and nutritional requirements for broodstock in seahorses. The only information available on lipid content and fatty acids profile was reported by Planas et al. (2008a, 2009a, 2010) and Álvarez et al. (2009) in eggs released by adults maintained in captivity and fed on adult *Artemia*. Lipid and fatty acid composition has been used as an indicator of egg quality in fish eggs because n-3 HUFAs are essential in marine fish (Sargent et al. 1989, 1999) and are largely affected by

the diet (Wiegand 1996). Generally, nutritional requirements are inferred from data obtained in the wild, especially on the biochemical composition of eggs and juveniles, but unfortunately this type of information is lacking (Lin et al. 2008c). In captivity, the highest content of fatty acids in eggs of *H. guttulatus* were 18:1n-9, 16:0, 18:2n-6, 20:5n-3, 18:0, and 22:6n-3, in decreasing order. However, it is difficult to predict to what extent this information could be extrapolated to other species as the biochemical composition of adult seahorses varies largely depending on species and origin (Lin et al. 2008a, 2008c), and the fatty acid profile in eggs can be easily modified by artificial manipulation of parental diet (Planas et al. 2009a, 2009b).

Skeletal malformations in early stages of marine fish might be due to nutritional factors (phosphatidylinositol, DHA, peptides, or retinoic acid) (Cahu et al. 2003). Severe episodes of mouth malformations (jaw deformities that impede normal feeding) accompanied by the release of premature juveniles have been reported in newborn juveniles of seahorses (Planas et al. 2009b). The problem was solved by increasing n-3 HUFA content in the diet (three- and fourfold increase in EPA and DHA content, respectively) and consequently in eggs (Planas et al. 2009a, 2009b). Newborn juveniles are usually released from the male pouch in one single batch. When released in more batches, during several successive days, the proportion of embryos and underdeveloped juveniles with yolk sac increased. This problem has been solved by isolating breeding males some days before the release of juveniles, as it was hypothesized that males may accelerate the release of young to be prepared again for mating.

Females are batch spawners with long inter-clutch intervals, which are dependent and synchronized with gestation duration (9–45 d) in males (Foster and Vincent 2004; Vincent and Sadler 1995). Optimal temperature for reproduction is unknown for the majority of seahorse species and needs to be determined due to its influence on gonad development and hatching. Interclutch interval in females seems to be controlled by temperature (Lin et al. 2006, 2007;

Planas et al. 2008b, 2010). Female maturation in *H. guttulatus* is dependent on the photoperiod regime, rather than temperature, which can boost up the effect of light regimes (Planas et al. 2009a). Temperature, and not photoperiod, was suggested as the main environmental factor governing reproduction in *H. capensis* (Lockyear et al. 1997). Photothermal manipulation has been successfully applied to shift the period of reproduction in *H. guttulatus* (Lockyear et al. 1997; Planas et al. unpublished data).

Rearing procedures with acceptable survival rates have been reported for *H. abdominalis*, *H. erectus*, *H. ingens*, *H. kuda*, *H. subelongatus*, *H. trimaculatus*, *H. reidi*, and *H. whitei* (Payne and Rippingale 2000; Job et al. 2002; González et al. 2003, 2004, 2006; Woods 2003a, 2003b; Wong and Benzie 2003; Ortega-Salas and Reyes-Bustamante 2006; Lin et al. 2006, 2008b; Wilson et al. 2006; Hora and Joyeux 2009; Murugan et al. 2009). Improvement in rearing methodologies during the past few years has contributed to significant increases in survival rates (20–90%) in *H. erectus* (50–90%) and *H. reidi* (20–85%) at commercial scale (Gomezjurado 2009a, 2009b).

Newborn seahorses are bigger than larvae of most marine fish species and sufficiently developed for active swimming and foraging activity. Newborns start feeding immediately after birth due to the lack of yolk reserves, ascending instinctively to the water surface to capture air for swim bladder inflation. In spite of these common characteristics, performance, prey preferences, and nutritional requirements vary largely among seahorse species, and adequate zootechniques must be established for each species. The rearing of juveniles is conducted differently for each species, with cultures being carried out in green or clear water, under natural or continuous light regimes, and with different types of prey enrichments. Juveniles of tropical and subtropical species are usually raised at 23–28 C, whereas species of temperate waters are raised at 13–24 C.

Feeding is one of the most decisive factors in the survival of juvenile seahorses (Alexandre and Simões 2009). The application of a universal feeding scheme suitable to all seahorse

species is not operative, as biology, size of newborns, and especially digestion capability are species dependent. Juveniles are fed on live prey and attempts to culture juveniles on inert, frozen, or dried food have been unsuccessful (Alexandre and Simões 2009). Rotifers are fed to juveniles of *H. reidi*, *H. kuda*, or *H. trimaculatus* (Garcia and Hilomen-Garcia 2009; Gomezjurado 2009b; Murugan et al. 2009). However, rotifers are not accepted by other species (low ingestion rates), being probably suboptimal for the energetic demand of juveniles of *H. erectus*, *H. hippocampus*, and *H. guttulatus* (Otero et al. 2007; Gomezjurado 2009a; Planas et al. 2009a). Copepods are an alternative/complement to *Artemia* and are known to enhance both survival and growth of cultured seahorses (Olivotto et al. 2008a, 2008b, 2008c; Hora and Joyeux 2009).

The effect of different types of water conditioning on survival and growth rate was examined for newly hatched *H. erectus* juveniles (Alexandre and Simões, unpublished data). Survival at 20 d was significantly larger in the “green” water treatment, when compared with “bio-floc” and the “clear water” control. Similarly, growth rate expressed in weight and length was significantly higher compared with the control. These results are explained as an extra nutritional load of *Artemia* metanauplii continuously feeding on the live microalgae present, together with a potential probiotic effect, through the regulation and/or stabilization of the system’s microflora, with a consequent positive effect on the juvenile digestive tract and feeding efficiency. The authors further hypothesized that the green color of the water induces different intensity and distribution of light through the aquarium, when compared with the “clear” water control, which minimized the concentration of food *Artemia* on the surface, reducing air bubbles formation and better consumption efficiency.

Some seahorse species are difficult to cultivate, probably due to two main factors: low digestive capacity during their early developmental stages and a high tendency to show swim bladder hyperinflation. In *H. abdominalis*, newborns are perfectly capable of digesting and

assimilating the food offered, including *Artemia* nauplii and metanauplii (Woods 2003a). Juveniles are also able to attach to a holdfast very soon after being released from the male pouch (Quintas, personal communication), reducing the risk of swim bladder hyperinflation. Newborns of *H. guttulatus* are also active hunters, but their capacity to attach to a holdfast develops 3–4 wk after birth, and their digestive capacity is extremely limited during the first week, especially when fed on *Artemia* nauplii or metanauplii (Álvarez et al. 2009; Planas et al. 2009a). Under these conditions, and accompanied by a progressive depletion of energetic reserves, juveniles ascend to the water surface, where the swim bladder hyperinflates due to the capture of air, stop feeding, and die in only a few days. Differences in digestive capability among seahorse species could be related to the lack of appropriate chitinolytic enzymes (e.g., *N*-acetyl- β -glucosaminidase; Álvarez et al. 2009; Quintas et al. unpublished data) and/or long digestion periods (Murugan et al. 2009). The digestive capability in early developmental stages can be improved by reducing gut passage time by dispensing prey in pulses and avoiding the permanent availability of prey (Planas et al. unpublished data). In such cases, copepods would constitute a suitable alternative to *Artemia* and/or rotifers (Olivotto et al. 2008a, 2008b, 2008c).

Different prey enrichment procedures used in the feeding of seahorses provide satisfactory results in terms of growth. In spite of the advances achieved in the rearing of some seahorse species, nutritional requirements in seahorses are unknown. Diets are commonly enriched in n-3 HUFA, which are essential to seahorses; however, requirements for n-3 HUFA and for protein have not been established yet. Nevertheless, the use of mixed diets is recommended to improve the nutritional status of the fish and to enhance the adaptation to non-living feeds (Alexandre and Simões 2009). The adaptation of juveniles to frozen food (mysids, *Acetes*, amphipods, and adult *Artemia*) is routine in the commercial production of seahorses

from day 30 to 70 posthatch (Gomezjurado 2009a, 2009b; Lin et al. 2009).

Although grow-out is commonly carried out in indoor tanks, interest for cultivating seahorses in cages is increasing in areas where adequate natural conditions are available. A good example is the effort for producing *H. reidi* in cages placed inside aquaculture ponds in Rio Grande (Brazil; Lima et al. 2009). Fish were raised with a minimum labor cost and fed on natural occurring food (copepods, amphipods, and caridean shrimp). The high survival rates achieved indicate good prospects for the extensive culture of seahorses.

An important source of juvenile mortality are diseases caused by protozoan infestations and pathogenic bacteria (*Vibrio* spp., *Flexibacter*, and *Mycobacterium*; Blasiola 1979; Alcaide et al. 2001; Tendencia 2004; Failde et al. 2008; Gomezjurado 2009b; Balcázar et al. 2009, 2010a, 2010b). Balcázar et al. (2010b) pointed out the dominance of Rhodobacteraceae (*Phaeobacter*, *Ruegeria*) in the cutaneous mucus of healthy adult seahorses. The bacterial communities recorded in the feces of adult seahorses were dominated by Vibrionaceae, being strongly influenced by the microflora of the diet (Balcázar et al. 2009). Given that the same bacterial patterns may also occur in juveniles, the application of disinfection procedures in live food may contribute to specific treatments for most generic diseases. However, further studies are necessary to determine which phylogenetic groups of bacteria dominate in the associated microbiota, as specific groups may play with variable physiological states (Balcázar et al. 2009, 2010b).

In conclusion, production of seahorses on a commercial scale is a relatively recent activity and there is good potential for culturing new species. Currently, tropical and subtropical species constitute the bulk of the commercial production of seahorses, using knowledge from research efforts on these species and other marine fish. However, not all seahorse species perform similarly under culture conditions due to interspecific differences in biological and physiological characteristics. This constitutes a limiting factor that restricts the

transfer of culture information among seahorse species, especially between those from tropical/subtropical waters to those of temperate waters.

Although available rearing technology promotes noteworthy survivals for some species, more studies are still necessary to optimize culture protocols, especially addressing nutritional and microbiological aspects. The understanding of feeding and nutritional requirements is of paramount importance for rearing success. In this way, the expansion of knowledge on the biochemical composition of natural prey and seahorse embryos/larvae (in the wild and in captivity) will significantly enhance our understanding of the nutritional requirements for breeding and rearing these highly popular marine ornamental fishes.

Marine Ornamental Invertebrates

Over 700 marine invertebrate species are currently traded in the marine aquarium industry (Wabnitz et al. 2003). Corals, both soft and stony species, are the most popular and most expensive group of marine ornamental invertebrates in the trade. Nonetheless, several groups of marine invertebrates are also heavily collected, such as other cnidarians (mostly sea anemones), mollusks (namely tridacnid clams and snails), decapod crustaceans (such as shrimp, crabs, and hermit crabs), and live rock (although not scientifically a marine invertebrate, it is commonly traded under the designation of Scleractinia, along with stony corals; Wabnitz et al. 2003). Some other groups of ornamental invertebrates that are also collected for marine aquariums, although in lower amounts, are polychaetes (namely tube-dwelling species commonly known as feather dusters) and echinoderms (such as brittle stars, sea stars, sea cucumbers, and sea urchins) (Sprung 2001; Shimek 2004).

Most marine invertebrates are traded for their dazzling colorations and delicate appearance (e.g., corals, tridacnid clams, and cleaner shrimp). However, a growing number of hobbyists currently buy several marine invertebrate species not for their coloration or

morphology but rather as members of “clean-up crews” employed by hobbyists as “aquarium janitors” to control the growth of nuisance algae and eat food leftovers (Sprung 2002; Calfo and Fenner 2003). In recent years, there has been a growing apprehension by researchers, policy makers, and conscientious marine aquarium traders, collectors, and hobbyists on how dependent the marine aquarium trade still is on the collection of wild specimens from coral reefs (Wabnitz et al. 2003; Rhyne et al. 2009a, 2009b). The global concern on the sustainability of this industry has promoted the need for culturing the most heavily harvested species. Making cultured specimens a suitable alternative to those collected from the wild has not been an easy task, mainly because of the lack of knowledge on the reproductive and larval biology of most traded species. This scenario resulted in several bottlenecks that have limited culture procedures from reaching commercial scale. Nonetheless, researchers and traders readily share the perception that the captive culture of marine ornamental invertebrates is more than a profitable venture, that it is in fact a need for the sustainable development of the industry. This synergy between academic and commercial goals has already resulted in the development of feasible culture protocols for several species (e.g., Ellis 1998, 2000; Calfo 2007; Calado 2008) and may well promote an increase in the number of species and specimens bred in captivity that will be made available for the marine aquarium trade (Calado 2009).

Live Rock

Live rock is the popular name that designates pieces of coral rock used in reef aquariums for functional (e.g., biological filtration) and aesthetic purposes. Live rock harbors a variety of invertebrates and algae (namely calcareous red algae, popularly known as coralline) as well as beneficial microorganisms (both nitrifying and denitrifying bacteria) that significantly improve water quality (Yuen et al. 2009).

Presently, live rock represents an important part of the revenue obtained by countries

exporting marine ornamental species (Wabnitz et al. 2003). However, the prolonged harvest of live rock is considered by most researchers as a potentially destructive practice, which may promote erosion and significantly decrease important fisheries habitats (Lovell 2001). In certain regions, the collection of wild live rock has already been banned (e.g., Florida, USA) or is being heavily monitored and legislated (Falls et al. 2003; Parks et al. 2003), which opens the window of opportunity for the aquaculture of live rock.

Live rock aquaculture may be as “simple” as providing an adequate substrate for the colonization of adequate microorganisms as well as highly prized benthic fauna and flora (namely incrusting coralline algae). Live rock can be produced onshore, employing flow-through or recirculating systems, or off-shore in open systems. Successful live rock onshore aquaculture facilities require large surface area and adequate light. In this way, most culture tanks are wide and shallow, with indoor culture facilities relaying on fluorescent or metal halide lamps to ensure proper light levels (required for the growth of photosynthetic organisms, namely coralline algae) and a source of heat (e.g., geothermal or electric energy) to ensure optimum water temperature. Concrete raceways, as well as fiberglass tanks are commonly employed in outdoor culture, with the shading of culture tanks being a common practice to avoid light and thermal stress. An erroneous assumption commonly associated with the production of live rock offshore is that once the rock is on the bottom, it will fully develop without any further intervention. In fact, the site must be regularly visited and inspected (at least once a month), namely after the occurrence of storms or hurricanes, to monitor excess algal cover and sedimentation (Falls et al. 2003).

Some aquaculturists choose to employ natural limestone rocks collected inland, whereas others exclusively use manufactured substrate, such as mixtures of limestone sand (aragonite), gravel, shells, and adequate cement (Falls et al. 2003). Despite the huge amount of anecdotal information available online on how to “cook your own live rock,” there is still a lack of

scientific studies comparing biological filtration efficiency of rocks prepared using different materials and/or methods. Robles-Gil et al. (2009) performed some preliminary trials using ecofriendly materials (white cement and sea sand on a 2:1 mixture, plus styrofoam balls [0.5-mm diameter] in different percentages [0, 15, 30, and 45%]) to prepare artificial live rock. The authors evaluated the degree of colonization of artificial rock matured under controlled conditions in an onshore flow-through system and compared the void volume, weight, and oxygen consumption among the different type of manufactured rocks using natural live rock as a control.

With the expected increase in airfreight price in the years to come, imported live rock will become even more expensive for marine aquarium keepers – shipping rocks by airplane will never be an inexpensive practice. Under this scenario, it is likely that traders may try to push the production of live rock closer to their target markets (e.g., by employing inland recirculating culture systems) and decrease shipping costs (taking it so close to the customer that may allow its shipment by land). This economic aspect, together with the growing restrictions that most countries are implementing toward the harvest of live rock, may well be the necessary boost that live rock aquaculture needs to become a more generalized practice among those culturing marine ornamentals.

Corals

Corals have been propagated asexually for several years, either by public institutions, private enterprises, or enthusiastic hobbyists. There are already a significant number of manuals on asexual coral propagation (e.g., Ellis 1999; Ellis and Sharron 1999; Shafir et al. 2006a; Calfo 2007). The improvements achieved in the fragmentation of certain reef-building corals allowed the production of large number of nubbins and boosted different fields of coral research, including aquaculture (Shafir et al. 2001, 2003, 2006a).

Corals have been cultured inland using recirculated systems, onshore employing flow-through or recirculating systems, and off-shore in bottom or suspended nurseries (Calfo 2007; Shafir 2006b, 2009; Shaish et al. 2008). The use of off-shore coral propagation structures is now also widely used by commercial enterprises, although these techniques were previously developed to help in the restoration of coral reefs (Rinkevich 1995, 2000). The use of mid-water coral nurseries is now a popular option, as it provides improved environmental conditions to growing coral fragments, namely optimized water flow, optimal light, no sediment accumulation, and a significant decrease in the risk of predation by corallivorous organisms (Rinkevich 2005). Another approach employed to promote the growth of coral fragments for coral reef restoration is the induction of mineral accretion (Hilbertz and Goreau 1996). This technology involves passing a low-voltage electrical current through a cathode and an anode, which induces electrolysis of seawater. This procedure triggers the accumulation of mineral ions dissolved in seawater within the vicinity of submerged electrodes and their deposition through electrochemical processes in the structure employed as the positive electrode (e.g., steel bars framework, and steel mesh; Hilbertz 1992; Sabater and Yap 2004; Borell et al. 2010). The electrochemical deposition of CaCO_3 or $\text{Mg}(\text{OH})_2$ in the cathode strengthens coral attachment (Sabater and Yap 2004), which is a feature known to enhance the chances in survival of transplanted corals (Ammar et al. 2000). This technique is yet to be applied in large scale for the production of stony corals for the marine aquarium trade, probably because of the existence of species-specific responses to this technique and the lack of consensual opinion on the suitability of this methodology to significantly improve coral growth and survival (Borell et al. 2010).

The fragmentation and growth of corals in recirculated systems located inland has been widely practiced both by private aquarium reef keepers, public aquariums, and commercial enterprises. The information available on the suitability of the propagation techniques

commonly used in this type of system is anecdotal and mostly relies on empirical, rather than scientific, knowledge. Some of the most relevant aspects that have been scientifically addressed concerning the propagation of corals in recirculating systems are the types of adhesives and substrates employed to attach coral transplants (Schlacher et al. 2007; Dizon et al. 2008), the influence of different light regimes (Reynaud et al. 2004; Schlacher et al. 2007; Schutter et al. 2008), and water flow (Khalesi et al. 2007).

For 12 species of corals studied, Dizon et al. (2008) showed that corals attached using cyanoacrylate glue detached significantly more from their substrates than those attached with epoxy putty or marine epoxy. Attachment experiments performed by Schlacher et al. (2007) with *Acropora solitaryensis* revealed that coral fragments grew equally well either in marble or cement bases, but metal halide lamps with a color temperature higher than 14,000 K promoted higher survival. The experiments by Schutter et al. (2008) on *Galaxea fascicularis* demonstrated that the enhancement of calcification only seems to be mediated by photosynthesis at lower irradiances, whereas at higher irradiances, skeletal growth is not limited by photosynthetic potential. Reynaud et al. (2004) demonstrated that *Acropora verweyi* fragments grew better under high light intensities and temperatures (400 $\mu\text{mol}/\text{m}^2 \text{ s}$ and 29 C) and that there was a strong correlation between growth rates and strontium uptake. The study addressing the effect of water flow on the growth performance and shape of *Simularia flexibilis* showed that this coral displayed morphological responses to shifts in water velocity and that the highest growth rate was achieved using a water velocity of 11 cm/s (Khalesi et al. 2007). It is important to highlight that all previous results should not be generalized for coral species other than those addressed in the described studies, as species-specific variations may occur for all studied parameters.

Threatened coral species (such as the elkhorn coral, *Acropora palmata*) are commonly cultured in captivity through fragmentation to restore natural populations as well as to

eliminate the need for public aquariums to harvest wild specimens to exhibit in their reef displays (Bruckner and Bruckner 2001; Delbeek 2001; Precht 2006; Raigoza Figueras et al. 2009). However, the use of asexually produced coral fragments to recover threaten species and/or promote reef conservation must take into account restoration genetics to be truly effective (Baums 2008). SEXual CORal REproduction was a pioneer project which applied sexual coral recruits on a large scale to stock public aquariums in a sustainable way (Petersen et al. 2006). Coral recruits were produced either from larvae released from colonies stocked in public aquariums or from larvae produced from gametes collected *in situ*. This approach can be an economical and sustainable alternative for supplying corals for marine aquariums as well as for coral restoration (Petersen et al. 2006, 2008a).

The need to transport coral larvae over large distances prompted research on this topic. Petersen et al. (2005a) described that over 90% of coral larvae shipped at densities less than 4 larvae/mL were able to survive for as much as 10 d of shipping, with best survival results being achieved when transportation time was less than 4 d. Larvae were shipped 4–6 d after fertilization in 10- μm -filtered seawater, without the addition of oxygen or any disinfectant (Petersen et al. 2005a). The development of ceramic tiles that maximized the settlement of coral larvae was another significant breakthrough in the propagation of sexual recruits (Petersen et al. 2005b).

Although the presence of biofilms (namely patches of incrusting coralline algae and algal turfs) is relevant for the settlement of coral larvae in a specific substrate (such as custom made ceramic tiles) (Petersen et al. 2005b), the physical properties of that substrate is also important. The presence of crushed coral rubble on cement tiles (at a concentration of only 10%) significantly enhances the settlement of coral larvae (Lee et al. 2009). These findings confirm the diversity of natural reef cues that can trigger settlement in larval corals (Heyward and Negri 1999). Petersen et al. (2008b) described how the simple addition of *Artemia nauplii* could

significantly enhance the growth performance of young sexual recruits and help them to be less prone to mortality during early postsettlement periods. The ongoing standardization on the production of coral sexual recruits will certainly reinforce the role that this approach is already playing toward the sustainable production of corals for the marine aquarium trade.

Anemones

Anemones have always been one of the most popular marine invertebrates traded for marine aquariums. The main reason for this popularity is certainly the symbiotic relationship displayed by anemones and clownfish (e.g., *Amphiprion* spp. and *Premnas biaculeatus*), as well as several other invertebrates (namely shrimp; Miyagawa 1989; Fautin 1991; Giese et al. 1996; Silbiger and Childress 2008; Mebs 2009). However, the most popular anemone species in the trade (e.g., *Heteractis* and *Stichodactyla* spp.) may be highly susceptible to overharvesting due to their long life span, slow growth rates, and low reproductive rates (Shuman et al. 2005). An additional aspect of concern is that the intensive harvest of clownfish, as well as symbiotic shrimp, for the marine aquarium trade may also negatively affect the survival of host anemones (Spotte 1996; Porat and Chadwick-Furman 2004, 2005). It is known that anemone cover plays a major role in the spatial distribution of clownfish (Richardson 1999); thus, it was not surprising for Shuman et al. (2005) to verify that the collection of wild specimens for the marine aquarium trade significantly affected the populations of anemones and their symbiotic fish. A clear example of the imbalances that an unregulated collection of anemones may produce is the absence of suitable habitat for new clownfish recruits to settle. As pointed by Shuman et al. (2005), the strict regulation of anemone collection would not only decrease the direct impacts on anemone populations but also decrease the current pressure on the populations of symbiotic anemonefish.

The need to manage the harvest of anemones and their symbiotic fishes and invertebrates

from the wild is more urgent than ever, as it is becoming clear that there is an interaction between coral reef decay and anthropogenic disturbance (e.g., collection for the marine aquarium trade; Jones et al. 2008). The growing awareness of the vulnerability of natural populations of anemones to overharvesting has urged conscientious researchers, traders, and hobbyists to advocate the captive culture of the most heavily traded anemone species. Currently, there are already records of successful asexual propagation of anemones in captivity by cutting healthy specimens longitudinally in half and attaching them to a suitable substrate (Calfo 2007; Centurión Fernández et al. 2009). However, no study has ever addressed in sufficient detail the optimization of asexual anemone propagation (e.g., determining the minimum size at which an anemone can be successfully propagated, the best attachment technique, and the suitability of different materials used in the attachment base).

It is also relevant to highlight that recent studies have provided a valuable amount of biological data on the reproductive biology (Scott and Harrison 2007a, 2009), embryonic and larval development (Scott and Harrison 2007b), as well as settlement and juvenile grow-out (Scott and Harrison 2008) of some of the most heavily traded anemones for marine aquariums (e.g., *Entacmaea quadricolor* and *Heteractis cripa*). Anemones used as broodstock were collected from the wild and kept in separate flow-through outdoor tanks. Broodstock tank outflow pipes were fitted with 250- μ m mesh panels employed to collect spawned gametes (Scott and Harrison 2007a). Spawned gametes were placed into 60-L plastic tubs filled with seawater and excess sperm was flushed from the tub according to the procedures described by Harrison (2006). The tubs were located indoors with a 12-h light:dark photoperiod, at a temperature ranging from 23.5 to 24.5 C and were continuously aerated with a slow stream of bubbles from Pasteur pipettes placed at each corner of the tub. This gentle aeration ensured not only water oxygenation but also suitable water circulation and overall water quality (as described by Harrison 2006). Larvae were cultured until settlement in 33-L

aquaria supplied with flow-through filtered seawater (approximately 0.6 L/min) and equipped with a settlement cage made of four, 250- μ m mesh panels in the sides and a biologically conditioned terracotta tile in the bottom for larval settlement (Scott and Harrison 2008). Cultured larvae were probably able to ingest nutrients dissolved in seawater, as well as particulate matter, and certainly received photosynthates from their endosymbiotic dinoflagellates (zooxanthellae). Aquasonic™ liquid invertebrate food (Aquasonic, Wauchope, NSW, Australia) was also provided to the aquariums employed in anemone larviculture and peak settlement and metamorphosis of planulae into primary polyps occurred 10 d after spawning (Scott and Harrison 2008). These promising results indicate that the sexual production of anemones to supply the marine aquarium trade may soon be a reality.

Polychaetes

Sabellid polychaetes, popularly known as feather dusters by marine aquarium keepers, are among the 10 top-most imported ornamental invertebrates (Wabnitz et al. 2003). These organisms deserve their popularity in the hobby due to their delicate appearance, stunning coloration of their tentacle crown, and their relatively large size. These tube worms, such as *Sabellastarte spectabilis*, commonly inhabit cracks and crevices in coral reefs, which makes their collection a challenging task. To harvest these organisms, collectors commonly employ destructive techniques (such as the use of crow-bars) that damage delicate corals and other organisms surrounding the tube worm. As a consequence, studies on marine ornamental tube worm reproduction and life cycle is of paramount importance for the establishment of suitable culture protocols (Bybee et al. 2006a). By culturing tube worms in captivity, it will be possible to decrease the fishing pressure on wild populations as well as contribute to the preservation of coral reefs by avoiding the use of destructive collecting practices (Bybee et al. 2009).

Presently, it is known that *S. spectabilis* is a protandric hermaphrodite (first maturing as

male during its life cycle and later as female; Bybee et al. 2006a). This species displays a long gametogenic period and an extended potential spawning season, which seems to be correlated with water temperature (maturation appears to coincide with water temperatures of 24–25 C; Bybee et al. 2007). As no significant correlation was found between day length and maturation stage of the tube worms, the same authors suggested that water temperature may be the most important factor influencing maturation and spawning.

Spawning induction trials already performed in captivity employed several aquaria assembled in a flow-through system at a temperature of 27 C. Some of the tube worms employed as broodstock were ablated (nearly 1 cm of the posterior end of their body was cut). This procedure may have triggered spawning, as it promoted the release of a chemical substance from the coelom that signaled other mature worms to spawn (Bybee et al. 2006b), as has been recorded for nereidid polychaetes (Hardege et al. 1998).

Fertilization is known to occur externally in *S. spectabilis*, with trochophore larvae emerging from egg cases and developing over 3 d before reaching the metatrochophore stage. By day 6–7, larvae are no longer competent to swim and settle on the bottom, where they start the construction of mucous tubes and adopt a sedentary life style by day 7–8. No larval food is required during this period, as *S. spectabilis* larvae are lecithotrophic (they rely on internal energy sources, such as yolk reserves, to fuel their larval development; Bybee et al. 2006b). Grow-out trials conducted in 60-L glass aquaria stocked with juvenile worms during 100 d revealed that these organisms were able to feed on a variety of live and preserved algal forms (live *I. galbana* [Tahitian strain], live *Nannochloropsis oculata*, tilapia greenwater, preserved *Isochysis* and *Nannochloropsis*; Bybee et al. 2009). Diets were provided twice per week at a density of approximately 1 million cells/mL. With the exception of preserved *Nannochloropsis*, all other tested diets promoted satisfactory juvenile growth and survival. The best results were achieved with

live and preserved *Isochrysis* (survival >80%), opening good perspectives for the commercial-scale culture of these organisms in the near future (Bybee et al. 2009).

Another species of tube worm commonly found in the Mediterranean and the Eastern Atlantic that has great potential for marine aquariums is *Sabella spallanzanii* (Calado 2006). The reproductive biology of these organisms is relatively well known (Giangrande and Petraroli. 1994; Giangrande et al. 2000), specifically after their introduction in Australian waters (Currie et al. 2000). The potential utilization of *S. spallanzanii* for the bioremediation of intensive aquaculture effluents revealed that these organisms can display remarkable growth rates (Giangrande et al. 2005). A significant biomass increment was also recorded by Pierri et al. (2006) for juvenile tube worms cultured extensively in suspended plastic nets commonly employed for growing mussel. Instead of using tube worms cultured extensively for bioremediation or as a potential feed, it will certainly be more profitable to sell part (or even all) of these specimens for the marine aquarium trade!

Mollusks

Giant clams in the genus *Tridacna* are unquestionably the most popular mollusks in the marine aquarium trade. These emblematic mollusks were once exposed to intensive fishing pressure (mostly for human consumption), which pushed wild populations to the brink of extinction and prompted their listing under Appendix II of the CITES (Bell et al. 2005). Fortunately, giant clams are one of the best examples of successful restocking programs addressing marine invertebrates (Bell et al. 2005, 2006; Gomez and Mingoa-Licuanan 2006). The possibility of inducing spawning in captivity, the short larval development of giant clams, and relatively low maintenance effort required during the grow-out of juveniles (part of their nutritional needs are satisfied by their symbiotic zooxanthellae) are some of the features that made these organisms highly suitable for aquaculture (Beckvar 1981). Giant

clam culture protocols are already well established for the most heavily traded species in the aquarium trade (e.g., Ellis 2000) and the current percentage of cultured specimens traded is certainly higher than the 20% reported by Wabnitz et al. (2003). At least in Europe, nearly 100% of traded giant clams are currently captive-bred and it is unlikely that this scenario may ever be reversed (imports are strongly surveyed by authorities and hobbyists already prefer cultured specimens, as these display higher survival in captivity).

Recent studies addressing the role that incrusting coralline algae may play in the settlement of larval *Tridacna* revealed that it may attract larvae but does not promote settlement in *Tridacna squamosa* (Neo et al. 2009). Another study by Lebata-Ramos et al. (2010) confirmed the potential of using giant clams produced in captivity for grow-out in ocean nurseries. However, this approach is unlikely to ever be implemented in European waters (or other places outside the natural distribution of giant clams) to supply the marine aquarium trade. Nonetheless, the grow-out of small captive-bred juvenile giant clams using closed recirculated systems is becoming more popular in Europe. This approach allows traders to import a large number of small-sized giant clams, grow them in captivity to larger sizes, and sell these large specimens for significantly higher prices.

Other bivalve mollusks are traded, although most starve to death on the aquariums due to their dependence on abundant phyto- and zooplankton food. A clear example is the caribbean fire clam or flame scallop, *Lima scabra*, that has been extensively fished in Florida, reaching landings higher than 65,000 specimens in 1994 (Rhyne et al. 2009a, 2009b).

The dazzling coloration of sea slugs makes them highly appealing for the marine aquarium trade. However, the strict feeding habits of the most appealing specimens (e.g., *Chromodoris* spp.) make them a poor choice, even for the most skilled hobbyists, as they will slowly starve to death in captivity (Sprung 2001; Calfo and Fenner 2003). Fortunately, a growing number of hobbyists are becoming aware that these organisms are better left in

the reefs and that only a reduced number of sea slug species may be successfully kept in marine tanks. One of those sea slug species is *Aeolidiella stephanieae* (still known in the trade by its former scientific name *Berghia verrucicornis*). This species is commonly employed to control the pest glass anemone, *Aiptasia* spp., as it feeds exclusively on this prey. This sea slug is very easy to breed, as it is a simultaneous hermaphrodite that readily spawns in captivity. The embryos hatch either as a juvenile (direct development) or as a lecithotrophic larva that does not require any food until metamorphosis (Carroll and Kempf 1990). Immediately after settlement (or hatching when direct development is displayed), young sea slugs start feeding on small glass anemones. The limiting factor to ensure a commercial-scale production of this sea slug is to ensure that enough glass anemones are available to sustain the voracious juveniles! Curiously, there is a shortage of *A. stephanieae* in Europe that could be easily overcome by enthusiastic hobbyists. The culture of another anemone-eating sea slug, *Spurilla neapolitana*, seems to be a less interesting alternative, as it has planktotrophic larva that require adequate feeding for at least 22 d (Schlesinger et al. 2009).

Another group of highly demanded sea slugs are the sacoglossans in the genera *Tridachia* and *Elysia*. These sea slugs are commonly employed to control the growth of nuisance algae (generally sold together with other “reef janitors”; Sprung 2002). These organisms have been intensively studied by the scientific community, as they retain functional chloroplasts from the algae they ingest (Rumpho et al. 2000, 2008). Several sacoglossans have already been raised in captivity for research purposes (e.g., Trowbridge 2000; Curtis et al. 2007; Rumpho et al. 2008), which suggests that published culture protocols may eventually be adapted to allow commercial-scale production.

Gastropod mollusks are commonly traded for marine aquariums as part of cleaning crews employed to control unwanted green algae, diatom biofilms, and food leftovers (Sprung 2002). Some of the most heavily traded species are those from genus *Trochus*, *Turbo*, *Tectus*,

and *Astraea*, with probably all traded specimens from these and related genera being collected from the wild (Wabnitz et al. 2003). It is interesting to point out that at least for some of these species (e.g., *Trochus niloticus* and *Turbo marmoratus*) there are already established large-scale culture protocols (Heslinga and Hillmann 1981; Murakoshi et al. 1993). Culture efforts have mainly addressed restocking efforts of threatened populations (Amos and Purcell, 2003; Bell et al. 2005), although the potential use of young juveniles for the marine aquarium trade has long been recognized (Bell and Gervis 1999). However, the lower value of the organisms commonly traded in “clean-up crews,” when compared with other marine ornamental species, makes their culture a less appealing activity (Calado 2009). Nonetheless, the increasing awareness of potential negative impacts associated with the collection of algae grazing species from coral reefs (Rhyne et al. 2009a, 2009b) may finally promote the appearance of cultured snails in the marine aquarium trade.

Decapod Crustaceans

The culture of marine ornamental decapods has experienced significant advances in the last decade, which allowed for the development of commercial-scale culture protocols for some ornamental shrimp and crab species (Calado 2008). The development of suitable maturation (Calado et al. 2007a), larviculture (Calado et al. 2003a, 2008; Palmtag and Holt 2007; Martinez et al. 2009), and grow-out systems (Penha-Lopes et al. 2005; Calado 2008; Pimentel and Calado 2009) was certainly the sign that private companies were waiting to address the culture of these highly prized organisms.

As previously stressed by Calado et al. (2003b), current efforts on marine ornamental decapods continue to be strongly biased toward the most-valuable species (e.g., ornamental shrimp in genus *Lysmata* and *Stenopus*), whereas the culture of some heavily collected species used in clean-up crews (particularly hermit crabs) has had little or no attention (Calado 2009). The potential for commercial-scale

culture of algae-eating crabs in genus *Mithraculus*, namely *Mithraculus forceps* and *M. sulphatus*, was already demonstrated in several studies (Penha-Lopes et al. 2005, 2006a, 2006b; Rhyne et al. 2005). However, the ongoing “competition” from inexpensive conspecifics collected from the wild does not make the culture of these crabs appealing for enterprises.

For algae-eating hermit crabs (e.g., those in genus *Calcinus* and *Clibanarius*), the present scenario is even more disappointing, as no study has ever addressed the suitability of these highly demanded ornamental decapods for culture in captivity. Available literature from ecological and behavioral studies indicates that suitable shells must be available for developing larvae to metamorphose (Oba and Goshima 2004), to maximize juvenile grow-out and reproduction (see review by Hazlett 1981). This need for a permanent supply of suitable shells appears to be the main bottleneck for algae-eating hermit crab aquaculture, as production costs would make cultured specimens just too expensive to compete in the marine aquarium trade with hermits collected from the wild.

A potential reason for the research bias toward high market value shrimp is the fact that reliable large-scale culture protocols are still missing for the most valuable species: *Lysmata amboinensis*, *L. debelius*, and *Stenopus hispidus*. Mated pairs of these species can be easily kept in captivity for long periods and produce consecutive batches of viable larvae (Calado et al. 2007a; Gregati et al. 2009a, 2009b). Curiously, this aspect has led researchers to neglect the relevance of maturation diets for marine ornamental shrimp broodstock maintenance in captivity. This erroneous assumption has promoted the production of poor-quality larvae, which can be easily ascertained by their poor survival during early stages of larviculture trials and contrasting biochemical profiles with those displayed by newly hatched wild larvae (Calado et al. 2009; Tziouveli et al. 2009). Providing a diversified maturation diet (e.g., a mix of enriched adult *Artemia* biomass, shrimp, mussel, Cyclop-Eeze® [Argent, Redmond, WA, USA], or squid) and feeding broodstock pairs

several times a day (six to eight meals) with small portions of the maturation diet (daily adding to 10% of the shrimp wet weight) seems to be a good starting point to produce large batches of embryos that may hatch as high-quality larvae (Calado 2008). The suitability of different live foods to newly hatched *Lysmata* is not consensual, with some researchers advocating the need to provide microalgae, rotifers, and/or copepod nauplii to newly hatched larvae, whereas others argue that newly hatched *Artemia* nauplii may be a suitable food even for the first larval stage (Simões et al. 2003; Palmtag and Holt 2007; Calado 2008).

The need to provide adequate prey in the first hours to newly hatched larvae is also far from being a consensual issue, as Cunha et al. (2008) suggested that newly hatched *L. amboinensis* would be able to survive 24 h of starvation and Calado et al. (2007b) suggests that similar periods of posthatching starvation significantly decrease larval survival. The study by Calado et al. (2005) demonstrated that even for *Lysmata* species, able to develop from the first to the second larval stage in the absence of food, it is extremely important to provide suitable feeding immediately after hatching to prevent mortality prior to metamorphosis as well as the occurrence of asynchronous settlement.

Culture trials of other popular ornamental decapods (excluding *Lysmata* and *Stenopus*) have been limited to a reduced number of shrimp species, namely *Thor amboinensis* and *Saron marmoratus*, which have been raised in commercial numbers by a Portuguese enterprise (Brian Schaff, personal communication). Martínez et al. (2009) have also reported the successful culture of *Periclimenes pedersoni*, although further studies are required to clarify the role of settlement cues by conspecifics and host sea anemones. The breakthroughs achieved by Martínez Pecero et al. (2009) in the culture of several marine ornamental decapods from the Gulf of California opens good prospects for the entrance of “new species” into the trade (e.g., *Palaemonella holmesi* and *Periclimenes lucasi*). The recruitment of new marine ornamental decapods for the aquarium trade that do not occur in the Caribbean or

the Indo-Pacific (e.g., Mediterranean species, such as *L. seticaudata*) may certainly help to alleviate the fishing pressure on some heavily collected species. Nonetheless, it is important to ensure that the specimens available from those new ornamental species are cultured in captivity and not collected from the wild.

Echinoderms

With the exception of the dazzling colored sea apples, *Pseudocolchirus* spp., and sea stars, echinoderms available in the marine aquarium industry are commonly traded as members of “clean-up crews.” Sea urchins, serpent stars (also known as brittle stars), and small sea cucumbers are collected in significantly larger numbers than any other echinoderms and are commonly employed by hobbyists to control unwanted algae, scavenge on uneaten food, and/or stir up sand beds employed in modern reef aquariums (Calfo and Fenner 2003). Given the importance that certain species of sea cucumbers and sea urchins play for human consumption and the urgent need to promote their conservation (Micael et al. 2009), there has been a growing effort toward the development of suitable culture protocols for the most commercially important species (e.g., Yokota et al. 2002; Hu et al. 2010). Despite the existence of relevant technical information on the culture of sea cucumbers and sea urchins, all specimens available for the aquarium trade are still collected from the wild. The progress achieved in the captive culture of the highly priced diadema sea urchin (*Diadema antillarum*; Idrisi et al. 2003), as well as of the green serpent star *Ophiarachna incrassata* (Fosså and Nilsen 2002), has not been enough to prompt the aquaculture of ornamental echinoderms. As already stressed for several other species employed as “janitors” in marine aquariums, the culture of these organisms is still regarded as not profitable, as long as the large number of specimens collected from the wild continue to out-price cultured ones (Calado 2009).

In conclusion, the number of cultured marine ornamental invertebrate species available for the aquarium trade has been slowly, but

steadily, increasing in the past few years. However, cultured specimens currently offered for sale are far too few to fulfill the growing demand displayed by this industry. With the exception of species listed under CITES (e.g., hard corals and giant clams), the supply of marine ornamental invertebrate species for marine aquariums still relies heavily on the harvest of wild organisms. This scenario is partly because of the relatively low market value at which some of the most heavily collected specimens are traded (e.g., species sold in clean-up crews). Their low market value makes them less appealing for enterprises raising marine ornamental invertebrates, which prefer to culture highly priced species (e.g., hard corals or ornamental shrimp). This trend is particularly noticeable for invertebrate groups with well-established culture protocols (e.g., algae grazing snails and echinoderms), where very little research effort would be required to successfully breed large numbers of specimens. Nonetheless, it is important to stress that adapting existing culture protocols for marine invertebrates used for human consumption to the culture of ornamental species may not be as straightforward as once assumed. The early optimism evidenced by Fletcher et al. (1995), toward the culture of marine ornamental decapod crustaceans by adapting established protocols for penaeid shrimp aquaculture, clearly demonstrated how illusive these assumptions can be. In fact, suitable protocols for mass rearing marine ornamental shrimp are still missing (Calado 2008). Unless collecting and/or importing restrictions are imposed on the marine aquarium trade, the low monetary value of many wild marine ornamental invertebrates will discourage and even impair the success of any commercial-scale venture targeting the culture of those species.

Sustainability and Traceability Issues

The sustainability of the marine aquarium trade is commonly questioned and this industry is often involved in controversy. Although traders try to maximize their profits, conservationists try to protect endangered habitats (namely coral reefs) and policy makers try

to mediate these conflicts by developing suitable legislation that may protect the marine environment, without impairing legitimate commercial activity (Wabnitz et al. 2003). The culture of marine ornamental species is commonly regarded as part of the solution of the marine aquarium trade sustainability issue but can accidentally also be the part of the problem. An example of this scenario is the harvest of wild postlarvae of fish and marine invertebrates for grow-out in captivity (Hair et al. 2004; Lecchini et al. 2006; Bell et al. 2009). The number and size of collected postlarvae, the by-catch of postlarvae from species with no value for the aquarium trade, and the effects of removing postlarvae from the ecosystem are some of the concerns expressed about the collection of marine ornamental species postlarvae (Bell et al. 2009). With the exception of small, isolated islands with limited postlarval recruitment, the responsible collection of postlarvae appears to be adequate for most locations and has insignificant negative impacts (Bell et al. 2009). The implementation of fishing strategies similar to those used for the collection of spiny lobster puerulus may be enough to reach “biological neutrality” (either by operating through a quota lease system and/or returning a number of juveniles to area of collection after grow-out in captivity to compensate for potential negative effects; Gardner et al. 2006).

Another pertinent issue is how captive-bred, captive-raised, and wild marine ornamentals can be distinguished from each other in a rapid and reliable way. To address this issue, the CITES Coral Working Group proposed the following source codes for hard (also known as stony) corals: “w” for wild, maricultured, or farmed corals (maintenance or growth of wild coral clippings or fragments in marine-based aquaculture systems); “f” for aquacultured corals (first-generation cultured corals produced in aquaculture systems); “c” for captive-bred or cultured corals (second-generation cultured corals produced in closed systems); and “r” for ranched corals (rearing of whole corals or larvae taken from the wild in a controlled environment; (CITES 2002a, 2002b). However, the absence of any morphological or biological

differences between cultured and wild corals makes their differentiation a challenging task. Potential marking solutions involving the use of barcodes attached to growing coral fragments (which will eventually be embedded in the coral skeleton as it grows) or the use of artificial dyes may not be sufficient, as there is still a lack of knowledge to recommend a reliable marking system (CITES 2002a, 2002b). Even with the implementation of ecocertification programs (such as those implemented by the Marine Aquarium Council for net caught fishes), the traceability of marine ornamentals along the chain of custody is not entirely reliable (Shuman et al. 2004).

Current solutions for the traceability of live fish used for human consumption (e.g., radio frequency identification tags; Hsu et al. 2008) are not adequate for marine ornamental fishes, as these “tagged” species (e.g., *Cobia* and groupers) are significantly larger than the majority of marine ornamental fishes traded in the aquarium industry. DNA barcoding solutions already implemented for the identification of marine ornamental fishes (Steinke et al. 2009) unfortunately cannot be used to distinguish cultured specimens from wild conspecifics. The culture of specimens displaying distinct colorations, which can make them easily differentiated from wild conspecifics (e.g., “snowflake” clownfish – predominantly white with orange blotches) may be an interesting way to identify marine ornamentals raised in captivity (Calado 2009). The aquaculture of species never before traded for marine aquariums may be a potential short-term solution to trace cultured organisms, although there is always the risk that, after a certain period, wild specimens will start to be traded under the label of “cultured specimens.” This scenario was already recorded for the Monaco shrimp *L. seticaudata*, a Mediterranean species that was introduced to the aquarium trade only in 2005. All specimens initially traded were raised in captivity, but only 1 yr after the start of its commercialization, wild conspecifics were already being traded as cultured specimens.

In conclusion, the sustainability of culturing marine ornamentals must take into consideration potential negative social impacts associated with culture efforts being centered in importing and not in the exporting countries, namely those in Southeast Asia and Central Pacific. As already stressed by Tlustý (2002), it is advisable that the know-how for the culture of marine ornamentals acquired by Western countries be shared with exporting countries, to provide an alternative to impoverished local populations currently depending on the collection of these marine organisms to survive. Tracing the origin of marine organisms is a huge challenge (Hastein et al. 2001; Moretti et al. 2003), tracing the origin of live marine ornamentals through noninvading and nondamaging techniques is currently an "impossible mission." In conclusion, there is an urgent need to develop innovative techniques that may allow traders, inspecting authorities, and hobbyists to reliably determine the origin of marine ornamentals.

Final Remarks

With the global decline of coral reefs, the aquaculture of marine ornamental species appears to be the most suitable alternative to the current pressures affecting these highly valuable organisms. The culture techniques presented in this article are an important update of the state of the art of marine ornamental aquaculture. Some of the most heavily collected marine ornamental species are already produced in commercial numbers. However, further research studies are still necessary to allow the regular supply of a broader number of cultured marine ornamentals in the marine aquarium trade. This study aims to disseminate the latest technical and scientific breakthroughs for the development of marine ornamental aquaculture and stimulate further research on the current bottlenecks still impairing the commercial-scale culture of several popular and pricey species. The major goal of marine ornamental aquaculture is not only to promote coral reefs conservation but also to develop a sustainable alternative to all those

involved in the collection and supply of these remarkable organisms to the marine aquarium trade.

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