AZA Egg Collecting and Larval Rearing

By Judy St. Leger, Gary Violetta and Eric Cassiano

Rising Tide Conservation was initiated by SeaWorld Parks and Entertainment in 2009 and has been embraced by many Association of Zoo and Aquarium (AZA) member facilities. The primary research site for this program is the University of Florida Tropical Aquaculture Laboratory (UF-TAL) in Ruskin, Fla. The program is dedicated to making breeding and rearing of marine tropical fish economically viable so that there are alternatives to collection. These techniques are critical for AZA collection sustainability. The program supports and directs research in marine fish production; including broodstock maintenance, spawning, larval rearing and live feed production, and grows out techniques. Regular communications with producers facilitates transfer of this technology into commercial production.

In 2010, a Conservation Endowment Fund (CEF) grant from the AZA gave the program a strong foundation of success. The grant was simple: aquariums would collect eggs from display tanks and ship them off to UF-TAL. The biologists there would feed the larvae, determine the species, and tell us all how to raise the fish. No problem. We all realize that "no problem" might have been a bit of an overstatement. After a reality check, we developed four specific questions we hoped to answer with this study:

1. Could we design collectors to catch pelagic eggs and deploy them in large display tanks? Many marine fish are pelagic spawners. Unlike demersal spawners, these fish release free-floating sperm and eggs into the water column to be taken away by the current. We needed to design collectors that could gently capture sufficient amounts of eggs prior to the rigors of filtration systems. Luckily, the pristine water quality encountered in most public aquaria reduced the amount of clogging the collectors would have to endure.

2. Could we ship the eggs without severely damaging them? Many marine fish eggs hatch very soon after release (~30 hours). Would the jostling during shipping injure the eggs or the larvae?

3. How would we determine what to feed the larvae? First feeds have been a major hurdle in rearing these species. Larval food preferences are based on a variety of factors including larval size and prey motility.

4. How would we identify the larvae as they were developing? Even after metamorphosis, many juveniles can look very similar. How soon could we know what we were raising?

With these specific goals in mind, we began producing and distributing egg collectors. Our initial project was slated to last one year and involved eight collaborating facilities. These included SeaWorld of Orlando, Florida Aquarium, the Columbus Zoo, Omaha's Henry Doorly Zoo, the Steinhart Aquarium, Virginia Aquarium & Marine Science Center, the John G. Shedd Aquarium, and the Georgia Aquarium. Egg collectors were designed based on airlift technology commonly utilized in aquaculture. They are not engineering masterpieces, but use simple technology to create a gentle water flow that skims the surface of the water and concentrates floating eggs inside a basket or tub. In the end, the preferred design involved a 1.5" diameter PVC standpipe with an airstone and water flowing into a basket with a 200 micron mesh. The design was kept simple so it could be easily duplicated and each individual aquarium could adapt it to fit the characteristics of their specific exhibits. It didn't take long to see results and begin collecting eggs. Once those initial shipments were received and reared, the excitement spread among the participating aquariums and some began to improve and implement their own collecting techniques. Although the majority still deploys floating collectors, some utilize other simple techniques such as nets placed in overflow sumps and hanging tiles (for demersal spawning species). As each species and each exhibit harboring them is different, often the best ideas for collecting eggs comes from the biologists who spend their days (and some nights) with these fish.

Since most marine fish spawn at dusk, collectors were deployed in the evening prior to lights out. In the morning, what was collected is transferred into a bucket. Collected eggs and debris were cleaned via a series of straining and settling to concentrate the eggs at the surface. While the eggs are floating to the surface, a shipping bag is filled with filtered tank water and oxygenated for five minutes by bubbling pure oxygen. The eggs are then placed into the bags for shipping. Sorting and cleaning viable pelagic eggs prior to shipping, as well as filtering shipping water, helps maintain water quality and improves transport. The bags were closed so that no air space remained. This step was an effort to reduce water sloshing during transit. Eggs were shipped via FedEX for next day delivery. Depending on the time of year and the origin of the shipment, cold or hot packs were included to help maintain temperature within the bag. Recently, studies to examine water temperature and its effects on incubation and hatching are being designed as cooler temperatures may actually aid in maintaining developing larvae. Determining the ideal density of eggs, water volume, and bag size are also topics of future research. Even though the speed bumps were numerous, we did have a significant amount of eggs that arrived in good condition at the Tropical Aquaculture Laboratory. As of this writing we have been able to successfully raise to metamorphosis Sea Bream (*Archosargus rhomboidalis*), French Grunt (*Haemulon flavolineatum*), Smallmouth Grunt (*Haemulon chrysargyreum*), Porkfish (*Anisotremus virginicus*), Bluestripe Grunt (*Haemulon sciurus*), Moonfish (*Monodactylus argentus*), Schooling Bannerfish (*Heniochus diphreutes*), Semicircle Angelfish (*Pomocanthus semicirculatus*), Orbiculate batfish (*Platax orbicularis*), Spadefish (*Chaetodipterus *faber*) and Ternate Damselfish (*Amblyglyphidodon ternatensis*).

Determining first feeds was a great challenge. The size diversity of marine fish larvae makes this an even greater challenge. Remember, the eggs for display aquaria come from multispecies tanks; shipments typically contained eggs from many species. Larvae often require species-specific culture protocols. Without knowing what is received, supplying the appropriate culture protocol is difficult. To address this challenge, the first food offered during this project was wild zooplankton; which includes copepods, ciliates, diatoms, tunicate larvae, polychaetes, tintinnids, and dinoflagelletes among many others. Wild zooplankton harvested from Tampa bay was provided to marine fish larvae. At that point, we would know what they were selecting as a first feeding diet and we could begin developing culture protocols for that species.

Another challenge within this project was the identification of eggs/larvae when they were received. Multiple species were often spawning within each exhibit. During the initial shipments we had no idea what we were receiving. However, as time went on and certain fish species grew to metamorphosis we were able to identify them. We used molecular identification techniques using DNA to identify fish larvae that did not survive to a state where we could identify them morphologically. One of the greatest advantages of using molecular techniques is that we discovered that we could get a positive identification from one egg. This made documenting eggs and larvae by photograph and later matching them up to the DNA results very useful. One of the problems is that molecular identification often took many days - too long to help with any particular shipment. However, as we began to document numerous eggs and larvae we were able to recall old photographs and quickly identify eggs and larvae based on previous shipments. The development of a marine fish egg/larvae catalogue is currently underway and will be extremely helpful in the examination of new species received.

The CEF grant for collection and shipping of eggs for AZA facilities has greatly advanced our abilities with marine fish aquaculture. Next steps in the Rising Tide program are all based on the foundations of this project. In 2012, we will set up specific marine species as broodstock to be able to perform repeated modifications of the egg collection, management, and larval rearing processes to improve and refine techniques. We will continue to catalog egg and larval morphology to make rearing easier. We hope to expand larval feeding with a better understanding of naturally selected foodstuffs. Rising Tide Conservation is based on collaboration. This collaboration is the strength of the program. If you'd like more information or to become an egg collection site, please check out our webpage (*www.risingtideconservation.org*) or our blog (*http://risingtideconservation.blogspot.com/*). We are interested in expanding the participating AZA facilities. We'd like you to join us.

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COMMENTARY

Acquisition of Fishes and Aquatic Invertebrates for Zoological Collections. Is There a Future?

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The majority of the freshwater fishes in the ornamental trade now originate from captive-bred sources, as do a large proportion of the freshwater species exhibited in public aquariums. In contrast, commercial operators who also supply marine specimens to the ornamental trade remove directly from the wild approximately 98% of the marine fishes and invertebrates exhibited in public aquariums. The common perception prevails that captive propagation is inherently a better alternative to obtaining animals from the wild. Although captive propagation has been shown to have many benefits for terrestrial species, there are a number of features unique to marine species that challenge the idea that every species should be bred in captivity. Some of the key issues relating to the development of widespread conservation-oriented captive propagation programs include: 1) the high taxonomic diversity in marine animals; 2) the resultant variety in their reproductive methods; 3) their ecological, behavioral, physiological, and nutritional needs; and 4) our general lack of knowledge on their husbandry and medical care. There are several characteristics of marine fish and invertebrate populations that make them suitable candidates for sustainable harvest. For instance, marine teleosts are "r-selected," meaning that they have an extremely high fecundity, and most marine teleosts have a wide distribution and the ability to disperse over long distances. In locations considered for fish collection, appropriate management techniques should be employed to ensure that fishes and invertebrates are collected with as little impact on the ecosystem as possible. The collection of marine fishes and invertebrates for public aquariums and the hobby

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trade should be managed like a fishery to ensure long-term sustainability. The public aquarium community should support marine organism certification initiatives, such as the Marine Aquarium Council (MAC). Marine organism certification will create market incentives that encourage and support quality and sustainable practices by creating consumer demand and confidence for certified organisms, practices, and industry participants. The creation of refuges that supply propagules to harvested areas, the rotation of areas fished, species-specific size limits and seasons, and standardization of collecting, handling, and transportation techniques should be used to manage these fisheries and harvest areas. Zoo Biol 22:519–527, 2003.

Key words: aquarium collections; fishes; aquatic invertebrates; collecting; sustainable fisheries; Marine Aquarium Council; MAC

INTRODUCTION

Over the last thirty years, the international zoo community has developed coordinated captive management programs for their living collections. These programs include the American Zoo and Aquarium Association's (AZA) Conservation Action Partnerships (CAPs), Taxon Advisory Groups (TAGs), Species Survival Plans (SSPs), and Population Management Plans (PMPs). These and similar programs were designed to ensure long-term species survival, as well as the availability of specimens for exhibit collections and education programs. Over the same period of time, advances in knowledge of husbandry, medical care, behavioral needs, and nutritional requirements have made the captive propagation of mammals, birds, reptiles, and amphibians commonplace. This trend has dramatically reduced the zoo community's dependence on the wild as a source of animals from within these taxa, so much so that over 86% of new mammals, over 65% of birds, and over 57% of reptiles and amphibians are now estimated to be captive bred (ISIS, 2000).

Increasingly diverse populations of captive-bred freshwater fishes have become available over the past few decades as a result of commercially driven aquaculture research and development activities to supply fishes for human consumption, restock recreational fisheries, and provide specimens for the ornamental fish trade. For example, most of the freshwater fishes in the ornamental trade now originate from captive-bred sources, as do a large proportion of the freshwater species exhibited in many public aquariums. In contrast, the majority (over 98%) of the marine fishes and invertebrates exhibited in public aquariums are usually removed directly from the wild by commercial operators who also supply marine specimens to the ornamental trade.

The common perception prevails that captive propagation is inherently a better alternative to obtaining animals from the wild. Although captive propagation has been shown to have many benefits for terrestrial species, there are a number of features unique to marine species that challenge the idea that every species should be bred in captivity. Some of the key issues relating to the development of widespread conservation-oriented captive propagation programs include: 1) the high taxonomic diversity in marine animals; 2) the resultant variety in their reproductive methods; 3) their ecological, behavioral, physiological, and nutritional needs; and 4) our general lack of knowledge on their larval husbandry and medical care. A considerable investment of resources and time, exceeding that given to terrestrial species, will be needed by public aquariums to develop this technology in order to meet the needs of their collections.

Andrews and Kaufman (1994) and Klocek (1995) provided an indication of the small number of existing captive propagation programs for fishes and some other aquatic animals at zoos and aquariums. According to the International Union of Directors of Zoological Gardens/Conservation Breeding Specialist Group of the International Union for the Conservation of Nature and Natural Resources, Species Survival Commission (IUDZG/CBSG (IUCN/SSC) (1993), critically endangered species should have a higher priority than less endangered species in captive breeding programs. The consensus of these groups is that preference should be given to those endangered species with which collaborating institutions have some husbandry and reproductive experience (thus providing a greater chance for success).

In recent years, the AZA's Marine Fishes, Freshwater Fishes, and Aquatic Invertebrate TAGs (among others) have been examining the role of captive collections in conservation, particularly the allocation of resources between ex situ and in situ efforts. At the core of these considerations are some fundamental questions: 1) In the absence of any substantial aquaculture efforts, to what extent can and should public aquariums support coordinated and widespread research on the captive propagation of marine species? 2) When, if at all, is the sustainable harvest of marine species an acceptable—even preferable—alternative for exhibit animals? 3) Should public aquariums invest considerable resources and time to breed everything in their collections? 4) How will public aquariums address current unsustainable practices associated with the aquarium trade, including the use of cyanide, overcollection, and associated habitat damage? These issues all stem from the problem of overfishing, which is the key issue to be addressed when examining the potential of sustainable harvests to meet the needs of public aquarium collections.

Challenges of Captive Propagation

In survey of North American zoos and public aquariums, Walker (1996) asked 51 responding institutions about their success in spawning and rearing marine fishes in captivity. In that survey, 41% of the respondents reported no success with breeding. When combined with institutions that achieved reproduction but failed to rear the offspring, the percentage of institutions that had no success in rearing any marine fishes increased to 63%. Of the 27% that reported successful breeding and rearing, the majority of successfully bred fishes were of three families: Pomacentridae (34%), Cottidae (12%), and Syngnathidae (5%). Walker reported that overall the efforts by zoos and public aquariums in rearing marine fishes were surprisingly poor and reflected a general lack of success within the industry. To understand the reason for this lack of success, one must look at the complicated early life histories of most marine fishes, and the often costly and labor-intensive investment needed to rear such species. Thresher (1984) organized reef fishes into five broad categories (categories 1–5 below) according to their reproductive strategies: we have included three other categories (6–8 below), the last two of which apply only to invertebrates:

1. Pelagic spawners. Release their sperm and eggs into the floating plankton community (this is by far the most widespread mode of reproduction on the reef).

2. Demersal spawners. Produce eggs that are typically site-attached, are often associated with a nest site (e.g., anemonefishes), or are mouthbrooders (e.g., cardinalfishes).
3. Egg-scatterers. Similar to pelagic spawners, but their eggs settle to the bottom (e.g., rabbitfishes and puffers).
4. Benthic broadcasters. Release pelagic eggs on the ocean bottom (e.g., moray eels).
5. Live bearers. Carry eggs in utero and release as free-swimming juveniles (e.g., brotulids, clinids, and embiotocids).
6. Pouch brooders. Brood eggs in a pouch and release free-swimming juveniles (e.g., syngnathids).
7. Asexual budding. Identical individuals or other life stages produced new individuals by budding (e.g., corals, jellyfishes).
8. Asexual fragmentation. Pieces are broken off and survive and grow (e.g. corals).

As illustrated by Walker's (1996) report, most successes in rearing marine fishes past metamorphosis by zoos and aquariums involved the demersal spawners with relatively large larvae, such as the pomacentrids (anemone fishes specifically), gobiids (gobies), brackish water cyprinodontids (killifishes), and cottids (sculpins). Of the pomacentrids, all successful rearing involved anemonefishes of the general *Amphiprion* and *Premnas*. Anemonefishes and a number of other demersal fishes are the most commonly reared, and many of these are available for sale through the aquarium trade. In large part, this success can be attributed to the fact that aquarists can easily access and monitor the eggs, and to the higher degree of parental care shown by those species. Larval fishes from demersal spawners are easier to collect by aquarists, and because of their large size, larval fish foods are easier to provide to them. Success with these species (e.g., anemonefishes) reflects the considerable effort given to developing culture methods because of the commercial potential and popularity of these species.

The success with the cottids as reported in Walker's (1996) survey can be attributed almost exclusively to the extensive propagation research efforts of the Vancouver Aquarium for the coldwater marine species of the Pacific Northwest. The Vancouver Aquarium's program represents the vast majority of successful rearings by a North American zoo or aquarium, and serves as an example of what can be achieved when sufficient effort and resources are directed towards a species or group of species. It provides insight into what might be achievable by aquaria, in terms of conservation and breeding, if they all took a similar approach. Syngnathids (seahorses and pipefishes) provide a unique opportunity for breeders to develop a pilot cooperative culture initiative amongst aquaria. The offspring of most species are born large enough to accept enriched, newly hatched, live brine shrimp (*Artemia*), a food item that most facilities can easily culture. Much is still unknown about completing the life cycles of these species in captivity. "*Project Seahorse*," an international conservation initiative (Hall et al., 1997) has been instrumental in organizing and focusing the efforts of public aquaria worldwide on documenting and standardizing culture methods for target syngnathid species, as well as related aquarium research, conservation initiatives, and education outreaches. Through a series of three workshops organized and facilitated by Project Seahorse in 1998, issues were identified and addressed ranging from sustainable harvest and socioeconomic considerations, to research and development of culturing methods. The John G. Shedd Aquarium, in partnership with Project Seahorse, created a dedicated Aquarium Research Coordinator (ARC) position specifically to facilitate implementation of actions prioritized through the workshops, and to help coordinate the collective efforts of public aquariums. This coincided with the prioritization of seahorses as a pilot culturing program by the AZA Marine Fishes Group (MFTAG). Seahorses can be raised in captivity, are threatened in the wild, and are a high-demand species in the trade (which is contributing to the species' decline).

Public aquariums have been successful with species that can asexually reproduce by budding or fragmentation. The Monterey Bay Aquarium, the Aquarium of the Americas, the Texas State Aquarium, and the New England Aquarium, among others, have been producing specimens of several species of jellyfish on a long-term basis. Once the environmental conditions are achieved, the jellyfish scyphistomae (polyps) continue to bud ephyrae (juvenile medusae). These animals often feed on the same foods as the adults, and are thus easier to care for than most fishes. Likewise, corals also reproduce readily by budding or fragmentation in captivity. Corals can be treated much like plants in that pieces can be broken off and attached to a new substrate. The Waikiki Aquarium, New York Aquarium, Columbus Zoo and Aquarium, Florida Aquarium, and Pittsburgh Zoo, among others, have successful coral culture programs (Brittsan, 1997). Even though some progress has been made with these taxa, the collection of specimens of most species will still be necessary to provide diversity for exhibit needs and supply animals for future culture technology research and development. Considering the vast diversity of invertebrates exhibited by public aquaria, it is not practical to assume that a well coordinated, successful culturing program could supply a significant percentage of the overall collection needs of public aquaria.

The breeding and rearing of pelagic spawners has been largely unsuccessful because of the lack of long-term efforts and problems with feeding and rearing the larvae. These species represent the fishes most often sought for acquisition by zoos and public aquariums for their collections, and include the angelfishes, butterflyfishes, surgeonfishes, wrasses, sea basses, and jacks. The culture of pelagic spawners presents a host of challenges. The first hurdle is collecting the eggs and larvae. Most pelagic spawners release their sperm and eggs into the water column shortly before or just after dusk. Most aquariums are designed with aggressive filtration systems to maintain a high degree of water quality, which quickly remove and destroy the floating eggs. Many reef fishes are exhibited in community tanks, where egg predation is a major issue. Coupled with the fact that reproduction usually occurs after most facilities have closed their operations for the day, the chances for successful collection of eggs and larvae are minimal. (It should be noted that the use of reproduction hormones may enhance the ability of pelagic spawners to breed). If successful collection occurs, the next challenge is providing a rearing set-up to house the small, fragile larval fishes while maintaining adequate water quality standards.

The greatest challenge for most aquaria is providing an adequate diet for the young fishes. In the wild, pelagic larval fishes feed upon phytoplankton, copepods, and other microscopic invertebrate larvae. In captivity, live plankton substitutes must be cultured and maintained if a program is to be successful. These cultures can include algal cultures (various species of unicellular algae), protozoa, rotifers, mysids, copepods, various marine invertebrate larvae, and live brine shrimp, often enriched with a fatty acid or yeast supplement. The lack of knowledge regarding the nutritional requirements of most fishes and invertebrates further complicates this situation. The larval organisms are microscopic and require live foods small enough to be ingested. As the larvae grow, a succession of larger food items must be continuously introduced. This requirement is further complicated by the large variation in growth rates among cohorts. In addition, the live food cultures must be provided in sufficient densities to minimize the effort expended by the larvae in searching for prey items. Maintaining the required densities of food creates water-quality problems that in turn lead to maintenance problems and disease. Success in dealing with these problems often requires the creation of a dedicated staff position, which may explain in part the small number of aquariums breeding marine fishes. Investment in staff expertise, labor, equipment, and facility space can be of critical importance in a successful breeding program.

Collection of Fishes From the Wild Will Be Necessary

Even with coordinated effort, the breeding of marine fishes and invertebrates will not fulfill the needs of public aquariums in the near future. Therefore, if public aquariums are going to continue to use animals in their exhibits and laboratories for education and research, the collection of animals from the wild will be necessary. A key question must then be addressed: is it ethical for marine fishes and invertebrates to be collected from the wild? Providing the following questions can be answered appropriately, there is no logical reason that fishes cannot be collected from the wild for public aquaria.

1. Are the species being collected threatened or endangered?
2. Is the habitat damaged in the process of collecting organisms?
3. Is the population being collected managed sustainably and not overfished?
4. Are the welfare issues of the animals being adequately addressed through collection, holding, packing, and transport to aquaria?

Very little is known about the wild status of most marine fishes. A three-day workshop in 1997 reassessed IUCN Red List criteria and applied them to candidate species. Consequently 113 species were identified as threatened, an increase from nine species listed in 1994. This suggests that many more marine species will be identified as threatened when more data become available. Only a fraction of all marine species have been assessed to date. Habitat degradation resulting from human activities such as deforestation (siltation), poor fishing techniques, and pollution (sewage and industrial waste) is a major cause of population decreases in the wild. Collection of species for aquaria is not a primary cause of reef destruction. In contrast, the ornamental fish trade is a high value per individual industry that, through training and ownership, can encourage communities to protect and maintain habitats through sustainable harvest.

There are several characteristics of marine fish and invertebrate populations that make them suitable candidates for sustainable harvest. 1) Marine teleosts are "r-selected," meaning that they have an extremely high fecundity. Many teleosts produce 10,000 to 1,000,000 or more eggs per year. 2) Most marine teleosts have a wide distribution. For example, the moorish idle and longnose butterfly occur throughout the Pacific Ocean from Australia to the Sea of Cortez. 3) Most marine teleosts have the ability to disperse over long distances. Although more research on this subject is needed, it is evident from the distribution patterns of many species that eggs and larvae are capable of traveling large distances over deep water to settle in isolated areas.

In locations considered for fish collection, appropriate management techniques should be employed to ensure that fishes and invertebrates are collected with as little impact on the ecosystem as possible. The current use of cyanide compounds and other destructive techniques in the aquarium trade must be actively addressed by public aquaria. The collection of marine fishes and invertebrates for public aquariums and the hobby trade should be managed like a fishery to ensure long-term sustainability. The creation of refuges that supply propagules to harvested areas, rotation of fished, areas, species-specific size limits and seasons, and other fishery techniques should be used to manage these fish populations.

Public aquaria and collectors must actively cooperate in the exchange of information about their respective industries and reach an understanding that helps each side meet its needs while ensuring that the environment is protected. Through the Coral Reef CAP (CRCAP), the MFTAG, and individual public aquariums, countries exporting aquarium fishes can be supported to develop sustainable aquarium fisheries. In an attempt to encourage better management of fisheries and educate the consumer, the AZA, several other conservation organizations, collectors, government/NGOs in the range countries, and the aquarium trade are currently involved in the creation of a certification program through the Marine Aquarium Council (MAC) that will establish standards for the collection, transport, and sale of marine fishes on an international basis. Public aquariums and hobbyists should be encouraged to acquire MAC-certified marine animals.

Supporting the collection of local fishes and invertebrates can benefit local economies by providing careers that are not environmentally destructive. For example, training local fishermen to hand-catch fishes with nets instead of using cyanide would help reduce the destruction of coral reefs. The International Marine Life Alliance has made tremendous strides in this arena (Barber and Pratt, 1997). In several instances around the world the creation of parks (mostly non-marine) has created jobs for people who may have otherwise been poaching animals or practicing other environmentally destructive means of making a living. The aquarium trade is relatively high-value business compared to the food-fish trade. It can be developed in a way that is beneficial to artesinal fishing communities, and is sustainable for both fish and invertebrate populations and habitat.

The collection of the cardinal tetra, *Paracheirodon axelrodi*, for the aquarium pet trade is a good example of a sustainably managed fishery. The cardinal tetra is an extremely common fish in the pet trade and can be found in any pet store. However, unlike most other tetras sold in the aquarium trade this species has not been bred in captivity commercially because of specific water-quality requirements for breeding. Dr. Ning Labbish Chao of the Universidad de Amazonas has been studying and monitoring the cardinal tetra fishery in the Rio Negro drainage. Because this fish has an extremely high fecundity, there is a surplus of fish produced each year that can be harvested for the ornamental fish trade. There has not been a decline in this population over the several years that this fishery has been monitored. Even though this example is of a freshwater species, it demonstrates that many of these very fecund fishes can be harvested sustainably without threatening the species as a whole, as long as the fish are closely monitored, and at the same time "environmentally safe" careers can be provided for fishermen.

CONCLUSIONS

If public aquaria are to lessen their reliance on wild populations to supply their collections, a commitment must be made to long-term research and development of culture methods. Priorities should be established to develop models for research on the lifecycles and rearing requirements of selected species. Work with the demersal egg-lying species with large larva should be continued. A number of species that have demersal nests and small larvae, such as the damselfishes of the genus *Dascyllus* and the triggerfishes (Balistidae), would provide good models for the breeding of other fishes. Although it is more difficult to collect the eggs and larvae of pelagic spawners, it is possible through the use of screens and settling basins. The use of sex hormones would facilitate the collection of eggs and larvae by making spawning events more predictable. A considerable amount of research will be needed to breed just a few of these difficult species. Public aquaria must develop the technical skills and apply resources to culture small live foods in order to be successful with fishes that have small larvae.

Although the commitment of institutional resources to achieve these goals will be costly, it is a venture in which zoos and public aquariums must invest. With proper management, these initial costs could return revenues if captive-bred organisms are sold in the trade. A movement within the industry is needed similar to that directed toward the culturing of jellyfishes and corals in recent years. Short-term benefits will yield increased scientific understanding of the early life histories of these species, many of which are unknown. Long-term breeding will help reduce our demands on wild populations to supply our collections, especially with species that may have restricted distributions or are under considerable fishing pressure. In addition, public aquariums could provide the acquired technology to aquaculturists interested in breeding ornamental marine species.

It is clear that public aquariums and commercial aquaculture facilities will not be able to supply all the species needed for their educational exhibits. Therefore, harvesting of marine species will be necessary to fulfill the needs of public aquariums in the future. Collecting in damaged or overfished habitats must be avoided, and the aquaria must work with collectors, other conservation organizations, the trade industry, and government/NGOs in the range countries to develop sustainable harvest and management strategies. Public aquariums can help educate and train the individuals who collect for them to prevent future damage to habitats, and to take a more active role in field conservation programs. A strategic plan should be developed by the MFTAG and the CRCAP to address these issues in a coordinated way that also considers conservation issues for marine species and their habitats. Certification programs created by the MAC will be extremely helpful in conserving this resource. If the harvesting of marine fishes and invertebrates is managed responsibly, it will benefit conservation efforts by supplying the animals that public aquariums need to educate the public, and at the same time support local fishermen by providing a long-term career that will not be detrimental to the environment.

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The dilemma of listing teleost fish species considered difficult to keep in public aquariums

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Identifying those species of teleostean fishes for which survival in captivity has been less than one year at any North American institution belonging to the American Zoo and Aquarium Association is one of the initial tasks undertaken by the recently formed Marine Fishes Taxon Advisory Group. This paper describes the rationale behind the development of that list and discusses the possible benefits, as well as potential liabilities, that such a list may yield.

The Marine Fishes Taxon Advisory Group of the American Zoo and Aquarium Association was officially sanctioned by the Wildlife Conservation and Management Committee in 1992. The membership of AZA includes a significant number of aquariums and oceanariums. The purpose of the Marine Fishes TAG is to identify and address the many marine conservation issues through the activities of AZA member institutions.

In comparison with other TAGs, the intended function of the Marine Fishes TAG is unusual because marine fish species are not generally considered endangered, at least not throughout their range. However, AZA and marine aquariums in particular can benefit greatly from including marine conservation issues in the AZA mission. One such issue concerns the pet trade in ornamental marine fishes, primarily coral reef fishes. Public display institutions need to consider the influence they have on market demand.

Aquarium fish represent a large proportion of the pet trade. All too frequently customers make inappropriate selections of pet fish through a lack of knowledge. Indirectly public aquariums are a major promotion for keeping fishes as pets and thus there may be an implied mandate for public education about issues related to this. Beyond public education aquariums may also have a responsibility regarding the choice of species for public display. If a public institution cannot succeed in keeping a particular species alive for any length of time, should these species be on display where visitors might be encouraged to acquire them as pets? For example, although staff at a public aquarium may know which butterflyfish species can be fed foods other than live coral polyps, by chance they may still acquire the 'less suitable' species, either when new stock arrives and substitutions have been made by the exporter or when a confiscated or refused shipment has been sent to the aquarium by customs or wildlife authorities. Thus, by a variety of routes, public institutions may be holding fish, such as certain *Chaetodon* species, which have never been successfully fed anything other than coral polyps. If such specimens are placed on display visitors may presume that there would be no problem in keeping that species. Granted that some home aquarists have expertise beyond that of some public institutions but such cases are exceptional.

One approach to accommodating a display of a difficult species is to place a pet advisory logo next to the identification label, together with a concise explanation of the particular problems in keeping that species (see Marliave *et al.*, this volume). The other solution is to avoid displaying such species, holding them off-exhibit for research on potential diets or other holding parameters. Research goals can be accomplished in a display setting but the prospects for misunderstanding the species' suitability as pets still remains unless interpretative graphics are used to explain the situation.

Provided that there is potential for a useful internal AZA listing of species difficult to keep alive, the question arises as to how such lists should be categorized. The Marine Fishes TAG has established a three-tiered system. The primary list includes only those species for which no AZA member institution has achieved successful holding for a period exceeding one year. One year has been arbitrarily adopted as a cut-off date for long-term starvation effects. Gradual starvation usually occurs within a few months for tropical reef fishes and given the number of fish species which have annual life cycles, an arbitrary requirement for successful holding beyond two or three years would necessitate determination of aspects of life history which simply are not available for many of the species which are available for exhibition. For longer time criteria to be adopted, exceptions would have to be made for short-lived species. A uniform requirement for keeping over one year thus seems both reasonable and conservative. Interestingly, there has been fair concordance in identifying those difficult species which have survived longer than a year; usually, two or more of the 14 Marine Fishes TAG members have reported the same experience with a species.

The secondary list consists of species which, although they will live in the aquarium for more than a year, require special care. They might still require pet advisory logos on their identification label graphics. This list will naturally be more contentious, because different institutions will have had different levels of success, but will ultimately prove useful for focusing husbandry research and disseminating husbandry information for verification at other institutions. Finalization of this list by the TAG has been deferred until the primary list has been established.

The final list consists of fishes which are easy to keep but may be undesirable in captivity for other reasons. It would include fish with toxic spines, aggressive species which cannot be placed indiscriminately in community tanks and species which can grow very large. Other possible reasons for listing may well exist, such as a marked potential for becoming established as an exotic nuisance species in the local waters in the vicinity of the AZA institution.

If endangerment in the technical sense of an IUCN or CITES listing should become an issue for marine fishes, overexploitation in the wild or habitat elimination could become the basis for developing a listing.

At time of writing discussions are centred on the primary listing. Valid objections have been expressed over any categorization of species on the primary list as being either impossible to keep or to the effect that they should never be displayed. Absolute terms should be avoided. Part of the dilemma is over whether the most difficult species should be kept in off-exhibit facilities or on display. Sometimes the best conditions for survival or for research exist in display rather than reserve tanks. Under such circumstances, however, it may be considered that an obligation exists to inform the visiting public that certain species on display are notably more difficult to keep than others.

Anxiety exists over whether an official listing might not be abused by outside parties wishing to place whatever restrictions possible on the keeping of wild animals in managed environments, that is, in captivity. There is further concern that members of the pet trade might construe an AZA listing as implying a need for restrictions on their trade. The hope is that some of the top North American pet wholesalers, who may have expertise which is different from that of most display institutions, will contribute on an advisory basis to the Marine Fishes TAG, in order that the most precise possible identification of difficult species may be achieved.

Trade restrictions introduced in Germany on the importation of certain groups of coral reef fishes have been made on a broad taxonomic basis, which is unfortunate because difficulties tend to exist on a highly species-specific basis. The opinion has been expressed that the Marine Fishes TAG should develop its own list of difficult species rather than to wait for some other party or group to impose broad restrictions on the availability of marine fishes.

Continued exploitation of wild fishes which never survive in captivity beyond a matter of months is a type of over-exploitation. Wild populations may not be affected by exploitation levels at this time but the demographic impacts of collecting are not well documented. It seems logical that rather than distributing all captured species the exploitation of those species which survive the longest under human care will, ultimately, amount to a lower overall exploitation rate.

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Advisory logos for pet-trade fishes in public aquariums

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As members of the Marine Fishes Taxon Advisory Group, three institutions, Vancouver Aquarium, John G. Shedd Aquarium, Chicago, and National Aquarium in Baltimore, have developed trial logos to advise visitors to public aquariums on the suitability of fishes for home aquariums. The objectives of the logos are explained and sample texts in use at the aquariums are given. The results of surveys on the reaction of visitors and pet keepers are briefly reported.

Public aquariums maintain a broad diversity of fish species representative of the full range of organisms in a particular zoogeographic or taxonomic grouping, according to the purposes of the exhibit. In certain types of exhibit, the species displayed may well not include any species regularly found in the pet trade but, in other cases, displays rely heavily on species which are commercially available. Tropical freshwater and coral reef exhibits, in particular, may incorporate species frequently occurring in the pet trade. Because exhibits in public aquariums may encourage the keeping of fishes as pets, aquariums have a duty to offer advice and education on this topic. The Marine Fishes Taxon Advisory Group (see Marliave *et al.*, this volume) has initiated efforts to undertake this aspect of public education.

Three AZA member institutions which are represented in the TAG, the Vancouver Aquarium, John G. Shedd Aquarium, Chicago, and National Aquarium in Baltimore, have developed pet logos for trial purposes. The logos are intended for infrequent use to highlight species which occur in the pet trade which are particularly suitable for home aquariums or, conversely, those which are less suitable and need careful consideration before being selected as pets. The objective is to inform visitors to the aquarium so that they will have the knowledge to ask the right questions when purchasing pet fish. These advisory logos do not indicate any complex hierarchy of difficulty in fish keeping but the simple distinction of easy versus difficult. Similarly, the logos do not comprehensively cover the full range of species available in the pet trade.

Indeed, it would be cumbersome to attempt to educate the visiting public about all the species on display which might merit advice regarding their maintenance requirements. Other measures are proposed to develop systems to advise prospective pet shop customers, such as the 'eco-labelling scheme' (Wood, unpubl.).

Our logos are intended for use in conjunction with identification labels on display tanks. Selected, concise text would be drafted for each specific label to accompany the advisory logo. In this sense, the logos are customized for each species by the accompanying text. The logos themselves, however, are intended to be stylized so that only two logical interpretations are possible: unsuitability by means of the international prohibition symbol of a red circle with an oblique slash across it or suitability indicated by the international laundry code symbol of a green square. Both logos surround the pet keeping symbol which might consist of a representation of a fish in a glass bowl (Fig. 1).

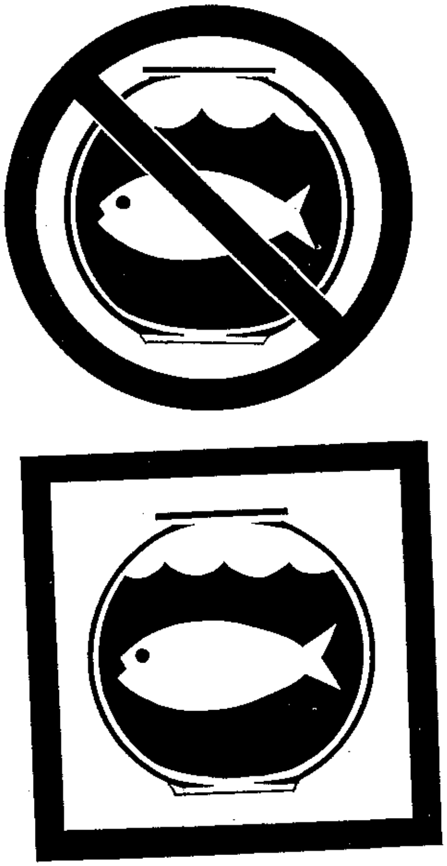

Fig. 1. Advisory logos are stylized to allow only two possible interpretations: unsuitability is indicated by a red circle with an oblique slash across it and suitability is indicated by a green square.

The accompanying text for the logos is intended to give pause for thought and to equip the visitor to ask pertinent questions when making a purchase. For example, at Vancouver Aquarium Yellow sailfin tangs *Zebrasoma flavescens,* Hawaiian damselfish *Dascyllus albisella* and Milletseed butterflyfish *Chaetodon miliaris* are labelled as 'suitable' pets with the following explanations under the green square logo: 'Most tangs or surgeon fish are hardy and easy to feed' (tangs); 'Fishes from Hawaii are net-caught. Net fishing is a preferred method because the fishes are healthier and it causes little damage to the environment' (damselfish); 'Most butterflyfishes *don't* make good pets because they need live coral in their diet! Milletseed butterflies are a rare exception: they eat readily available food' (Milletseed butterflyfish). All three species are displayed in close proximity to several others which were selected to illustrate the potential problems associated with the keeping of coral reef fish. The 'less suitable' species selected and displayed with the red circle logo are Lionfish *Pterois volitans:* 'Lionfishes must be treated with extreme care: they have toxic spines!'; Polkadot grouper *Cromileptis altivelis:* 'Polkadot groupers are often caught with cyanide. Cyanide eventually kills not only the fish, but also the coral reef where it was caught. *The fish in this exhibit were net-caught!';* Mottled moray eel *Gymnothorax undulatus:* 'Unless you have an aquarium as big as this exhibit, most moray eels will outgrow your tank.'.

These examples of potential and 'less suitable' pet fish species are in adjacent display tanks, with an overall introductory graphic in the identification label rack 'KEEPING FISH AT HOME? DO IT RIGHT! Although the fishes you see here are beautiful, it's not easy to tell which of them would make good pets! If you're thinking of keeping a fish at home, you must consider: its environmental needs—water quality, temperature, habitat; its compatibility with other fish—territoriality, lifestyle, etc.; its food requirements; its full grown size; whether it's captive-bred or wild-caught; how it was caught; and whether it's endangered and why.'. Next to this text are the two pet logos, with the underlying text 'Look for these symbols, beside some of the fish identification labels, in these two exhibits. They help to illustrate a few of the above points.'.

At the National Aquarium in Baltimore, three green-square logos indicating suitability as pets are for goldfish 'Goldfish are a domesticated form of a wild species and are quite hardy and well suited to life in the home aquarium', African cichlids, 'Many of the tropical freshwater fish that are available from pet stores are now bred on special fish farms and are no longer removed from the wild. Ask your pet store about captive-bred fishes' and by another African freshwater display, 'Many tropical freshwater fish can thrive in the home aquarium, but always check their requirements before purchasing them.'. Examples of the red-circle logos are for groupers and moray eels, 'Some of the fish offered for sale in pet stores grow very large and are not well suited to the home aquarium', live coral, 'Many of the marine invertebrates offered for sale in pet stores require very specialized care. Always check out their requirements before purchasing' and Pacific coral reef fish, 'Some of the marine fishes offered for sale in pet stores are quite difficult to care for. Only choose hardy species for the home aquarium, and discuss their requirements with knowledgeable pet store staff before purchase.'.

At the Shedd Aquarium, background surveys were conducted with a target audience of those who already are consumers in the pet trade for tropical marine fish and invertebrates. Two interns independently conducted a survey of visitors and interviewed 62 home aquarists. This was followed by a second survey of 50 customers at a local pet retail shop, using an identical format. A third mail survey targeted 53 individuals who had taken fish husbandry classes at the Aquarium. The last survey also included several different examples of possible logos, including those described, both with and without accompanying descriptive texts. The information collected indicated that roughly half of the consumers surveyed were dissatisfied with the information they received from pet shops on selection and care of specific animals. A minority of respondents in each of the surveys indicated that they sought information from books, libraries, fish care magazines or advice from friends, although those who had attended a course were over twice as likely as other respondents to seek information. Over 80% of respondents stated that they would be interested in seeing information on public aquarium graphics about the suitability of fish for home tanks. The responses regarding logo types were not amenable to analysis but generally indicated variable comprehension without the presence of explanatory text suggesting that further explanation is desirable. For example, with the logo for unsuitability, the Shedd Aquarium has the heading text 'Not suitable for home aquariums' with a terse explanation below, such as 'Grows too large'.

There are many possible ways to deploy pet advisory logos in display identification label racks in order to stimulate visitors to become wiser consumers. Only a few strategically selected examples need be highlighted with one of the advisory logos and accompanying specifically tailored texts in order to achieve the goal of increasing consumer awareness about keeping aquarium pets. The members of the AZA Marine Fishes TAG believe that providing these logos constitutes a worthwhile conservation effort on the part of public display institutions, which goes some way towards educating their visiting public to some of the issues regarding the keeping of aquatic life as domestic pets.

ACKNOWLEDGEMENTS

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REFERENCE

Wood, E. M. (Unpublished): *Trade in tropical marine fish and invertebrates for aquaria: proposed guidelines and labelling scheme.* An unpublished report for the Marine Conservation Society, 1992.

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An easy and effective device for breeding anemone fish *Amphiprion* spp at Stuttgart Zoo Aquarium

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Although the Aquarium at Stuttgart Zoo has bred and exhibited young anemone fishes *Amphiprion* spp for over 25 years, one of the problems of breeding marine fishes, the sensitivity of the larvae of some species to changes in water quality, remained. In 1989 the Aquarium developed a breeding tank which provides a simple method of separating newly hatched larvae from the breeding pair while maintaining the same water system in both sections of the tank.

Comparatively few species of marine fishes are successfully bred in aquariums, the main difficulties being the need for plankton as food, particularly during the first few days of life, and the sensitivity of the larvae of some species to changes in water quality. The Aquarium of the Wilhelma Zoological and Botanical Gardens

Effects of Aquarium Collectors on Coral Reef Fishes in Kona, Hawaii

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Abstract: *No previous studies have conclusively documented the magnitude of the effect of aquarium collecting on natural populations. In Hawaii concern over the effects on reef fish populations of collecting for the aquarium trade began in the early 1970s, primarily in response to multiple-use conflicts between aquarium-fish collectors and recreational dive-tour operators. In 1997–1998 we used a paired control-impact design to estimate the effect of aquarium collectors. We compared differences in fish abundance along visual belt transects between collection sites, where collecting was known to occur, and control sites, where collecting was prohibited. To test the assumptions of our observational design, we surveyed a combination of species captured by aquarium collectors and those not captured. The extent of bleaching, broken coral, and coral cover was also surveyed. Seven of the 10 aquarium species surveyed were significantly reduced by collecting. The abundance of aquarium fish at collection sites ranged from 38% lower (*Chaetodon multicinctus*) to 75% lower (*C. quadrimaculatus*) than that at control sites. In contrast, only two of the nonaquarium species displayed a significant collection effect. There were no significant differences in damaged coral between control and collection sites to indicate the presence of destructive fishing practices. In addition, there were no increases in the abundance of macroalgae where the abundance of herbivores was reduced by aquarium collecting. Although our results suggest that aquarium collectors have a significant effect on the abundance of targeted aquarium fishes, better knowledge of the intensity and location of collecting activities is required to make a rigorous assessment of the effects of collecting on nearshore fish populations. Several lines of evidence suggest that the current system of catch reporting underestimates actual removals.*

Efectos de Colectores de Acuario sobre los Peces de Arrecifes de Coral en Kona, Hawai

Resumen: *La magnitud del efecto de la recolección para acuarios sobre poblaciones naturales no ha sido documentada concluyentemente en ningún estudio previo. La preocupación por los efectos de la recolección para el comercio de acuarios sobre las poblaciones de peces de arrecifes comenzó a principios de los años 70 en Hawai principalmente en respuesta a los conflictos de uso-múltiple entre colectores de peces para acuarios y operadores de viajes de buceo recreativo. En 1997–1998 utilizamos un diseño apareado de control de impacto para estimar el efecto de colectores de acuario. Comparamos diferencias en la abundancia de peces a lo largo de transectos visuales en sitios de recolección, donde se sabía que ocurría recolección, en relación con sitios control en los que la recolección estaba prohibida. Para probar los sopuestos de nuestro diseño observativo examinamos una combinación de especies capturadas por los colectores de acuario y otra de especies no capturadas. Se examinó también la extensión de blanqueo, coral roto y cobertura de coral. Siete de las 10 especies de acuario examinadas estaban reducidas significativamente por la recolección. Las abundancias de peces de acuario en sitios de recolección variaron de 38% menos (*Chaetodon multicinctus*) a 75% menos (*C. quadrimaculatus*) individuos que en los sitios control. En contraste, sólo dos de las especies no recolectadas para acuario mostraron un efecto significativo de recolección. No hubo diferencias significativas en el coral dañado entre los sitios control y de recolección que indiquen la presencia de prácticas pesqueras destructivas. Además, no hubo incrementos en la abundancia de microalgas donde la abundancia de herbívoros se redujo*

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por la recolección para acuarios. Aunque nuestros resultados sugieren que los colectores de acuarios tienen un efecto significativo sobre la abundancia de los peces de su interés, hace falta un mayor conocimiento de la intensidad y localización de las actividades de recolección para evaluar rigurosamente los efectos de la recolección sobre las poblaciones de peces costeros. Varias líneas de evidencia sugieren que el sistema actual de registros de captura subestima las remociones reales.

Introduction

Global trade in ornamental fishes is a major industry involving approximately 350 million fish annually with a value of $963 million (Young 1997). Although marine fishes account for only 10–20% of the total ornamental catch, rapid increases in the collection of marine species occurred in the 1980s (Andrews 1990). Moreover, whereas freshwater fishes are largely derived from cultivated stocks, <1% of marine fishes are cultivated, and the majority are taken from wild populations (Wood 2001). Almost all marine ornamental fish are of tropical origin, and many are removed from coral reefs. Because aquarium-fish collectors are highly selective and often capture large quantities of individuals of high value, the potential for overexploitation is high (Wood 1985, 2001).

Although numerous authors have discussed the potential effect of the aquarium trade on marine fishes in Australia (Whitehead et al. 1986), Djibouti (Barratt & Medley 1990), Hawaii (Taylor 1978; Walsh 1978; Randall 1987), Indonesia (Wood 1985), the Philippines (Albaladejo & Corpuz 1981), Puerto Rico (Sadovy 1992), and Sri Lanka (Edwards & Shepherd 1992), few studies have estimated the effects of collecting on natural populations. The most common approach has been to examine the rate of international trade (Lubbock & Polunin 1975; Wood 1985; Andrews 1990; Edwards & Shepherd 1992; Young 1997). Other approaches include qualitative or quantitative observations of fish densities in collected areas (Albaladejo & Corpuz 1981; Barratt & Medley 1990) or comparisons of collection rates to crude estimates of sustainable yield based on field estimates of density (Edwards & Shepherd 1992). Although Nolan (1978) concluded that aquarium collectors did not have a significant effect on natural populations in Hawaii, the results are suspect because of problems with suitable controls in the observational design. Thus, no study has conclusively documented the magnitude of aquarium collecting on natural populations, despite repeated calls for such studies to help develop sustainability in the aquarium trade (Walsh 1978; Wood 1985; Young 1997).

Many of the marine ornamentals originating from the United States are captured in Hawaii, which is known for its high-quality fishes and rare endemic species of high value (Wood 1985). Concern over the effects of aquarium collecting on reef fish populations arose in the early 1970s, principally for the Kona coast of the island of Hawaii (Taylor 1978; Walsh 1978). Controversy has centered on multiple-use conflict between aquarium-fish collectors and recreational dive-tour operators over apparent declines in nearshore reef fishes (Taylor 1978; Grigg 1997; Young 1997; Clark & Gulko 1999). These concerns prompted the Hawaii Division of Aquatic Resources (DAR) to instigate monthly collection reports from all permit holders in 1973 (Katekaru 1978), and these reports have been the primary basis for management of the aquarium industry in Hawaii (Miyasaka 1994, 1997).

Based on collection reports, about 90,000 fish, with a reported total value of $50,000, were harvested in 1973 under 75 commercial permits (Katekaru 1978). In 1995 the annual harvest had risen to 422,823 fish (total value of $844,843) under 160 commercial permits (Miyasaka 1997). Although aquarium collecting was primarily centered on the island of Oahu in the 1970s and 1980s, the Kona and Milolii areas of the island of Hawaii became the predominant collecting areas in the late 1980s and early 1990s. Between 1993 and 1995, the harvest from Kona increased 67% and accounted for 59% of the state harvest with 47 commercial permits (Miyasaka 1997).

Although 103 fish species were collected statewide in 1995, over 90% of the harvest was focused on 11 species: the Achilles tang (*Acanthurus achilles*), Potter's angelfish (*Centropyge potteri*), raccoon butterflyfish (*Chaetodon lunula*), multiband butterflyfish (*Chaetodon multicinctus*), ornate butterflyfish (*Chaetodon ornatissimus*), four-spot butterflyfish (*Chaetodon quadrimaculatus*), goldring surgeonfish (*Ctenochaetus strigosus*), longnose butterflyfish (*Forcipiger flavissimus*), clown tang (*Naso lituratus*), moorish idol (*Zanclus cornutus*), and yellow tang (*Zebrasoma flavescens*), with *Z. flavescens* accounting for 52% of the total collection (Miyasaka 1997; DAR, unpublished data). Thus, given the increasing rate of removal focused on a small number of species, the potential for overexploitation of these reef fishes is high.

In addition to the direct effects of collecting fish for the aquarium trade, there has been considerable concern about destructive practices associated with fish capture. These practices include the use of poisons and explosives to capture fish and damage to coral during collecting (Lubbock & Polunin 1975; Wood 1985, 2001; Randall 1987; Johannes & Riepen 1995; Young 1997). An additional concern is the effect on the coral reef community of large reductions in the number of herbivorous fishes, such as the yellow tang. Because herbivorous fishes may control the abundance of algae on coral reef ecosystems, their removal may cause shifts in community structure (reviewed by Hixon 1997).

Our goal was to obtain quantitative estimates of the effects of aquarium collectors on fishes on the Kona coast of Hawaii. Moreover, in response to reports of broken and bleached coral associated with destructive fishing practices, we also investigated changes in the associated coral reef habitat at each study site.

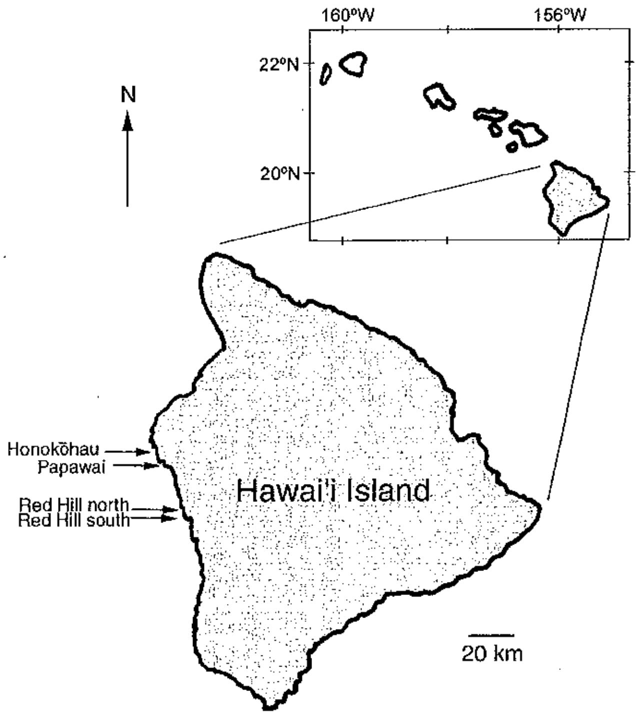

Figure 1. Map of study sites located off the island of Hawaii.

Methods

Observational Design

We used a paired control-impact design to estimate the effect of aquarium collectors on reef-fish abundance. The magnitude of the effect was estimated by comparing fish abundance at collection sites where aquarium-fish collecting was known to occur with geographically adjacent control sites where collecting was prohibited. Because the study was initiated after collection had begun, we assumed there were no differences between control and collection sites in the abundance of aquarium fishes prior to the onset of aquarium harvesting (i.e., their natural abundances were similar) (Osenberg & Schmitt 1996). We also assumed that all differences between the control and collection sites were due to aquarium-fish collecting and not other factors, such as fishing. As part of our study design, we gathered data to test these assumptions.

We established four study sites that served as two replicate control-collection pairs (Fig. 1). One pair of study sites was located at Honokohau (lat 19°40.26′N, long 156°01.82′W) and Papawai (lat 19°38.83′N, long 156°01.38′W). Papawai, a fishery management area (FMA) where collection of aquarium fishes has been prohibited since 1991 (Department of Land and Natural Resources 1996), served as our control site. Honokohau was frequented by aquarium collectors and served as a collection site. This pair of sites is hereafter referred to as the Honokohau study area. The second pair of sites was located at Red Hill North (lat 19°32.90′N, long 155°57.74′W) and Red Hill South (lat 19°30.32′N, long 155°57.17′W). Red Hill South is an FMA where the collection of aquarium fishes has been prohibited since 1991 (Department of Land and Natural Resources 1996), and it served as our control site. Red Hill North was frequented by aquarium collectors and served as a collection site. This pair of sites is hereafter referred to as the Red Hill study area.

At each study site, four permanent 50-m transect lines were established at 10- to 15-m depths by installing stainless steel eyebolts at the beginning and end points of each line. Transects served as reference lines for both the fish and coral surveys. We used a visual strip-transect search method to estimate fish abundances (Sale & Douglas 1981). A pair of divers swam side by side down either side of the transect line and counted all fishes seen within a corridor 3 m wide and extending to the surface.

Surveys began at Honokohau in March 1997 and at Red Hill in September 1997 and ended at both areas in December 1998. All sites were sampled at 2- to 5-month intervals, for a total of eight surveys at Honokohau and five at Red Hill. During each survey we estimated the abundance of 21 fish species. These species included 11 aquarium fishes selected on the basis of high levels of capture, accounting for over 92% of the fish collected in Hawaii (DAR, unpublished data). Due to uncertainty in species identification, we pooled longnose butterflyfish as *Forcipiger* spp., which may include both *F. longirostris* and *F. flavissimus*, although most of the fish counted were probably the latter (personal observations). The remaining 10 fish species we surveyed were not targeted by aquarium collectors but were in guilds similar to those of collected species. These species were selected to provide tests of the assumptions of the observational design. Although the assumption of no difference between the control and collection sites prior to the study could not be tested directly, one prediction of this assumption was that uncollected species should not differ between control and collection sites. Accordingly, *Acanthurus nigrofuscus, A. nigroris, A. triostegus, Chaetodon lunulatus, C. unimaculatus, Paracirrhites arcatus, P. forsteri, Plectroglyphidodon johnstonianus, Stegastes fasciolatus*, and *Thalassoma duperrey* were also surveyed. The overall structure of the fish communities at control and collection sites should also be similar if the sites are ecologically similar. Thus, to test this prediction, during the next-to-last survey at each site all reef fishes seen were counted and identified to species.

Of the 21 species surveyed, 2 species (*C. lunula* and *C. unimaculatus*) were too rare for analysis, with one individual of each species observed during the entire study. These species were excluded from further analysis.

Divers were undergraduate students who had completed a rigorous coral reef monitoring course and were trained in species identification and standardized survey methodology (Hallacher & Tissot 1999). To minimize observer bias, the same diver pairs were used at each control-collection study site during each survey. Divers did, however, vary among surveys. To minimize temporal variation, all surveys were conducted during midday (generally from 0900 to 1500 hours), and both control and collection sites were surveyed either on the same day or on consecutive days.

To provide an additional test of similarities between control and collection sites and to test for destructive harvesting methods associated with aquarium collecting, we also conducted surveys on corals, macroalgae, and the general substratum of each transect. Divers took photographs of the substratum with a Nikonos V camera with a 15-mm lens attached to a PVC quadrat covering an area of approximately 0.50 m^2 (0.8 × 0.6 m). Along each 50-m transect line, 18 photographs were taken at randomly selected coordinates at all study sites at both the beginning and end of the study. Percent cover estimates were made of all living and nonliving substrata in each photograph by projecting the slide over a series of 50 random coordinates and recording the observed substratum under each point. In addition, the percent cover of bleached and broken coral was estimated for each slide. We identified broken coral as recently damaged coral fragments with no algal overgrowths. We identified bleached coral as unusually pale portions of the coral colony located at the tips or edges of coral colonies. To minimize observer bias, a single observer analyzed all the photographic data.

Data Analysis

We analyzed fish data with two-way repeated-measure analysis of variance (ANOVA). Fixed factors included control and collection study sites ("effect"), replicate study areas (Honokohau and Red Hill or "area"), and the interaction between effect and area. Although each survey provided an estimate of the level of collection through control-collection differences, because the same individual fish may have been counted between surveys, surveys were treated as a random, repeated measure in the analysis (Zar 1996). A significant "collection" effect indicates a similar difference between control and collection sites at both study areas. A significant "collection-area" effect indicates a difference between control and collection sites that varies between study areas. A significant "area" effect indicates spatial differences in abundance among study areas. Because our goal was to obtain estimates of the magnitude of collection effects, only factors associated with a significant collection effect were interpreted (e.g., only collection or collection-area interactions, not temporal variation).

We calculated the percent difference in abundance as the difference between control and collection sites using the formula

$$\text{percent difference} = \frac{D_{\text{collection}} - D_{\text{control}} \times 100}{D_{\text{control}}},$$

where D is density expressed as number of individuals per 100 m^2. Thus, a negative percent difference associated with a significant collection effect indicates the presence of significantly fewer fish at collection sites than at control sites, whereas a positive value indicates the opposite.

We analyzed coral cover, bleaching, and breakage data with a three-way ANOVA, with effect, area, and time (beginning of study vs. end of study) as fixed factors. Data from photoquadrats along transects were treated as a random nested factor.

Prior to all analyses, we examined data for homogeneity of sample variances. We used transformed data in cases where the original data demonstrated heteroscedasticity. We did not examine normality because samples were small ($n = 4$) and normality is not an important assumption for ANOVA (Box 1953). Following ANOVA, we used the procedure described by Underwood (1997) to pool nonsignificant factors.

We used species richness (S), evenness (J), and the Shannon-Wiener composite diversity index (H') to examine overall fish and coral-algal-substratum community structure. We compared community structures by using the percent similarity index (Krebs 1986). These indices tested the prediction that the overall structure of the fish and coral-algal-substratum communities at control and collection sites would be similar.

Results

There was a significant difference in the abundance of aquarium fishes between control and collection sites but no differences in the abundance of nonaquarium species between these sites (Table 1, Fig. 2). Seven of the 10 aquarium species displayed a significant collection effect in the two-way repeated-measure ANOVA. In contrast, only two of the nine nonaquarium species, *P. arcatus* and *S. fasciolatus*, displayed a significant collection effect (Table 1, Figs. 3 & 4).

Of the 10 aquarium species, three exhibited a significant collection-only effect (Fig. 3). All of these species

Table 1. Mean (SE) percent change in fish abundance between sites with aquarium-fish collection and without aquarium-fish collection for each study area.

		Percent change[a]								
		overall		*Honokohau*		*Red Hill*		*p*[b]		
Species	*df*	*mean*	*SE*	*mean*	*SE*	*mean*	*SE*	*effect (E)*	*area (A)*	*E * A*
Aquarium species										
Chaetodontidae										
Chaetodon multicinctus	1,88	−38.2	6.57	−42.0	9.05	−32.3	9.63	0.02	-	-
Chaetodon ornatissimus	1,88	−39.5	20.2	−37.0	25.8	−43.4	36.4	-	<0.01	-
Chaetodon quadrimaculatus	1,87	-	-	−94.4	4.81	21.8	94.7	0.01	<0.01	-
Forcipiger spp.	1,86	-	-	−60.9	6.20	−43.6	19.5	0.01	<0.01	0.01
Pomacanthidae										
Centropyge potteri	1,87	-	-	−29.2	15.8	−73.1	12.3	0.03	<0.01	-
Acanthuridae										
Acanthurus achilles	1,88	−57.1	10.2	−64.0	13.3	−46.0	16.3	<0.01	-	-
Ctenochaetus strigosus	1,88	−14.7	8.20	−33.6	4.96	15.4	9.65	-	-	-
Naso lituratus	1,88	31.2	34.2	66.5	50.8	−25.2	25.1	-	-	-
Zebrasoma flavescens	1,87	-	-	−49.8	6.89	−43.2	6.47	<0.01	<0.01	-
Zanclidae										
Zanclus cornutus	1,88	−46.5	11.9	−45.9	16.1	−47.5	19.2	<0.01	-	-
Nonaquarium species										
Cirrhitidae										
Paracirrhites arcatus	1,86	-	-	−12.1	14.1	−75.3	3.16	<0.01	<0.01	<0.01
Paracirrhites forsteri	1,88	58.4	59.3	168.3	85.7	−73.6	14.5	-	-	-
Chaetodontidae										
Chaetodon lunulatus	1,88	−70.0	10.4	−70.0	10.4	-	-	-	-	-
Pomacentridae										
Plectroglyphidodon johnstonianus	1,88	−31.3	12.6	−12.1	15.2	−61.9	14.2	-	-	-
Stegastes fasciolatus	1,87	-	-	488	281	50.0	22.4	0.04	<0.01	
Labridae										
Thallasoma duperrey	1,88	17.4	12.4	31.6	17.0	−5.3	13.2	-	-	-
Acanthuridae										
Acanthurus nigrofuscus	1,87	27.3	22.8	15.2	26.7	46.7	43.5	-	<0.01	-
Acanthurus nigroris	1,88	67.2	63.6	−18.0	36.7	186.5	140.0	-	-	-
Acanthurus triostegus	1,88	−4.26	20.8	−5.68	32.4	<0.10	<0.10	-	-	-

[a] *A negative mean percent change indicates fewer individuals at effect relative to control sites.*

[b] *The* p *values and degrees of freedom (df) are reported for a two-way repeated-measure ANOVA on density.*

displayed a similar significant difference between control and collection sites at both study areas in which individuals were significantly more abundant at the control sites. These species, and the magnitude of their overall percent difference at collection sites, were as follows: *A. achilles*, −57%; *C. multicinctus*, −38%; and *Z. cornutus*, −47% (Table 1). (The negative percent indicates fewer individuals at collection than at control sites.)

Four species exhibited a significant collection and area effect (Table 1; Fig. 4). These species displayed significant differences between control and collection sites, but their overall abundance varied between study areas. Both *C. potteri* and *S. fasciolatus* were more abundant at Honokohau than at Red Hill, whereas *C. quadrimaculatus* and *Z. flavescens* were more abundant at Red Hill than at Honokohau (Fig. 4). The magnitude of their overall percent difference (in parentheses) at collection sites were as follows: aquarium species: *C. potteri*, −56%; *C. quadrimaculatus*, −75%; *Z. flavescens*, −46%; nonaquarium species: *S. fasciolatus*, +64% (Table 1).

Two species exhibited a significant collection-area interaction effect, where differences between control and collection sites varied between study areas (Table 1; Fig. 4). In the aquarium species *Forcipiger* spp., percent difference was greater at Honokohau (−61%) than at Red Hill (−44%). In contrast, the nonaquarium species *P. arcatus* displayed a lower percent difference at Honokohau (−18%) than at Red Hill (−75%) (Table 1; Fig. 4).

The overall fish community structure of the paired control and collection sites was remarkably similar. The H' diversity index at control and collection sites, respectively, was 1.18 and 1.16 at Honokohau and 1.16 and 1.17 at Red Hill. Similarly, the evenness index at control and collection sites, respectively, was 0.72 and 0.69 at Honokohau and 0.69 and 0.69 at Red Hill. At Honokohau, 44 species were seen at the control site, whereas 48 species were seen at the collection site. Forty-nine species were observed at both control and collection sites at Red Hill. Overall fish densities were 27% higher at Red Hill (mean density = 146 fish/100 m^2) than at Honokohau

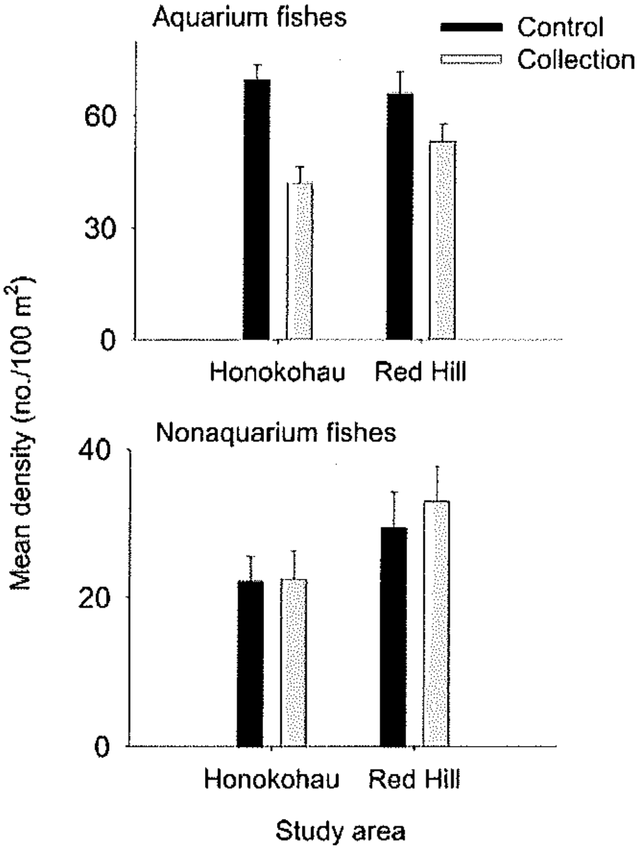

Figure 2. Mean fish density (±1 SE) for pooled aquarium and nonaquarium species at control and collection sites in both study areas.

(107 fish/100 m^2). Accordingly, control-collection pairs exhibited higher percent similarity (0.85-0.88) than that among study areas (0.75).

Live coral cover was significantly different between control and collection sites and between initial and final surveys, and there was a significant collection-survey interaction (all $p < 0.05$; df = 1,566; Fig. 5). Coral cover at all sites increased an average of 2.8% per year and was similar at both Honokohau sites but higher at the collection than at the control site at Red Hill. At Red Hill, coral cover increased 4.6% at the collection site and 2.3% at the control site (Fig. 5).

The amount of bleached coral was significantly different among areas ($p < 0.01$; df = 1,561): mean cover of bleached coral was 2.8% at Honokohau and 4.6% at Red Hill (Fig. 5). No other factors or interactions were significant. The percent cover of broken coral exhibited a significant difference among surveys ($p = 0.01$, df = 1,559): the mean cover of broken coral was 12% at the beginning of the study and 17% at the end (Fig. 5). No other factors or interactions were significant.

The abundance of macroalgae was low at all sites. No macroalgae was seen in the photoquadrats at Honokohau, and cover was <0.01% at the Red Hill sites. In contrast, coralline algae was fairly common at all sites.

The overall coral-substratum community structure of paired control and collection sites was similar. Species diversity, evenness, and richness were similar at all sites, and control-collection pairs exhibited higher percent similarity in community structure (79-82%) than that among study areas (63%).

Discussion

Seven of the 10 fishes targeted by the aquarium trade were significantly lower in abundance in areas subjected to collecting than in areas where collecting was prohibited. The magnitude of these differences ranged from −38% for *C. multicinctus* to −75% in *C. quadrimaculatus*. In contrast, only two of the nine nontarget species were significantly less abundant in collecting than in control areas, bolstering the conclusion that aquarium collectors have significant effects on the abundance of targeted fishes on the Kona coast of Hawaii.

Evaluation of Assumptions

The most critical assumption made when estimating the effects of differences between control and collection sites is that the parameter of interest is similar at both sites prior to the effect (Osenberg & Schmitt 1996). Otherwise, spatial variation in initial abundance can confound control-effect differences. For example, Nolan's (1978) study on aquarium collectors compared a collection site from the Kona area to a control, or "seldom-collected" site about 30 km away in north Kohala. His conclusion that collectors have no significant effect on abundance was based on finding a greater number of aquarium fishes at the collection site than at the control site. However, given the large distance between control and collection sites and the fact that aquarium collectors operated at both sites, this conclusion is unwarranted because of the high potential for confounding spatial variation with potential human effects.

Pairs of geographically adjacent sites minimize spatial variation, but this potential problem remains for all control-effect designs if there are no data prior to the onset of the effect (Osenberg & Schmitt 1996). Although the assumption of no prior differences cannot be tested explicitly, it can be inferred from several lines of evidence, including examination of spatial variation in fishes that are ecologically similar but not subjected to collecting and comparisons among the habitat of both sites. To evaluate this assumption, we used a combination of nontarget species that were ecologically similar to target species, species that were indicators of particular habitats, and examination of the coral habitat.

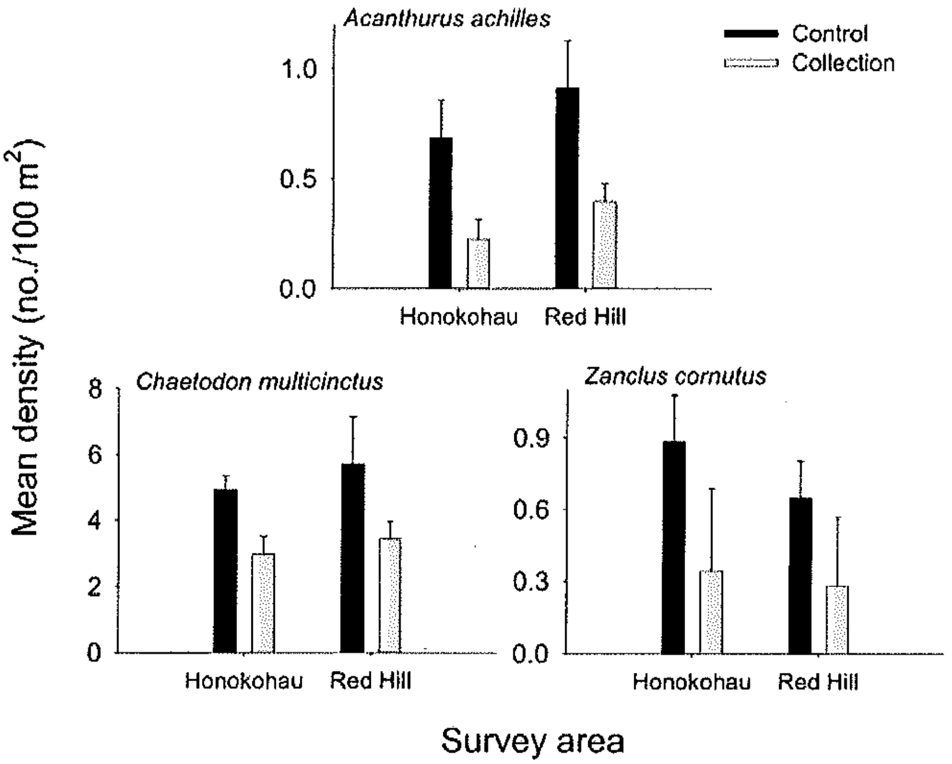

Figure 3. Mean fish density (±1 SE) for aquarium species that displayed significant collection-only effects. These three species are targeted by collectors of aquarium fish.

For example, the nontarget brown surgeonfish (*A. nigrofuscus*) and the targeted yellow tang (*Z. flavescens*) are both generalized herbivores that feed on filamentous algae, occupy the same depth ranges and habitats, and exhibit similar patterns of spawning and larval recruitment (Randall 1985; Walsh 1987; Lobel 1989). Yellow tangs were 47% less abundant at collection than at control sites, whereas brown surgeonfish did not differ significantly between the sites. Similarly, no differences were observed between control and effect sites among species that feed or live in close association with coral (*C. lunulatus, P. johnstonianus*), whereas their targeted counterparts (*C. multicinctus, C. ornatissimus, C. quadrimaculatus*) exhibited significantly lower abundances at effect sites. Moreover, nontarget species with generalized diets and distributions across the reef (*A. nigroris, A. triostegus, P. forsteri, S. fasciolatus, T. duperrey*) also did not vary, whereas ecologically similar aquarium species (*A. achilles, C. potteri, Z. cornutus*) were significantly different.

An additional line of evidence supporting the assumptions of our observational design is that the overall fish community structure of control and collection sites was remarkably similar in species diversity, richness, and evenness, with the percent similarity index ranging from 85% to 88%. At the habitat level, control and effect sites were also similar with respect to the diversity of coral, algae, and nonliving substratum composition, with percent similarity ranging from 79 to 82%. Thus, at several levels there was considerable support for the assumption that the reef communities were similar at both control and effect sites.

Another important assumption is that differences in abundance between control and effect sites were due to aquarium-fish collecting and not other processes that selectively affect these species, such as fishing. We addressed this assumption by selecting collection sites largely inaccessible from shore, thereby minimizing the effects of shore-based fishing. Moreover, both the aquarium fish *C. strigosis* and the nontarget species *A. triostegus* are commercially and recreationally fished in Hawaii. However, *A. triostegus* did not vary significantly between control and effect sites, indicating that fishing impacts were not significantly different in these areas.

Illegal collecting at control sites would also confound control-effect differences. Although some illegal collecting may be occurring in Kona, it is probably uncommon and unlikely to have a significant effect on fish abundances in existing protected areas (W. Walsh, personal communication). Thus, the only clear difference between the control and effect sites in this study was aquarium-fish collecting, as evidenced by the significantly lower abundance of aquarium species at the collection sites.

Indirect Effects of Aquarium Collecting

Destructive practices associated with the collection of fish are common and include breaking coral to capture

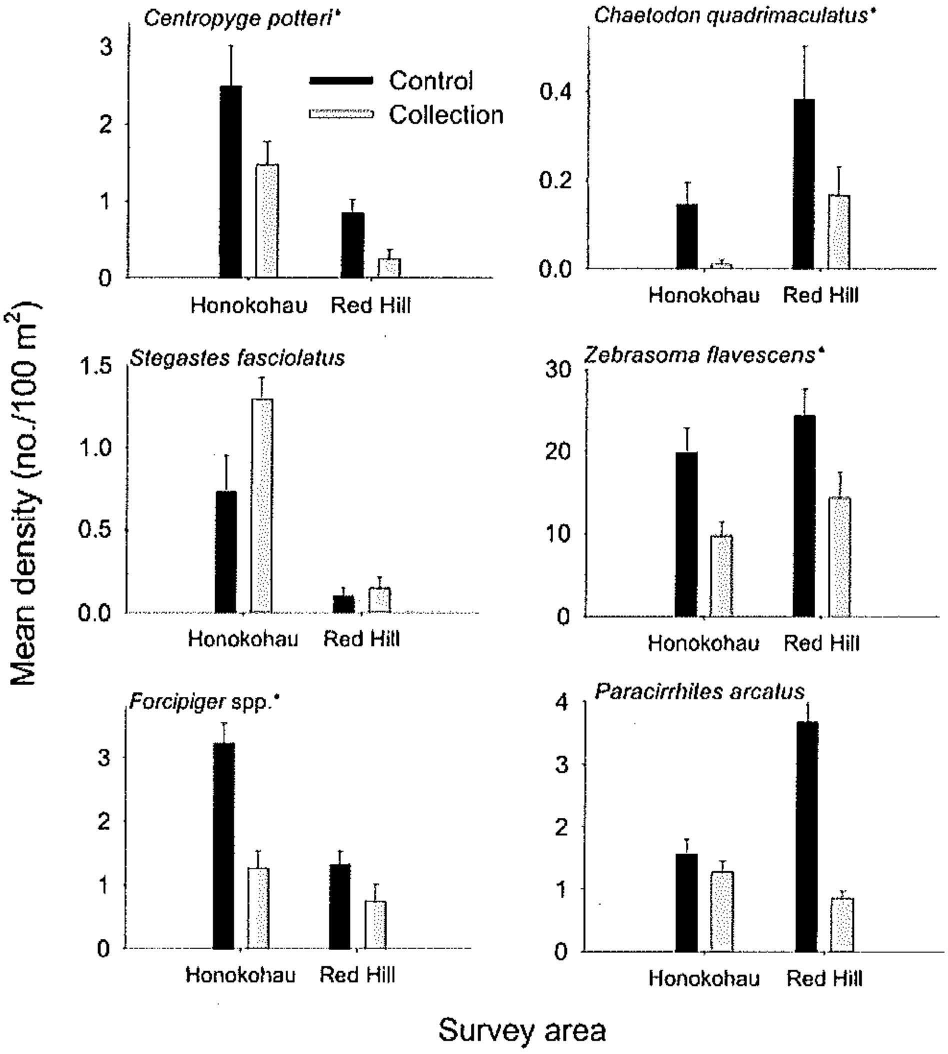

Figure 4. Mean fish density (±1 SE) for aquarium and nonaquarium species that displayed significant collection, area, or collection-area interaction effects. Species targeted by aquarium collectors are indicated with an asterisk (*).

live animals, snagging nets on coral, and using bleach and cyanide to stun target species (Randall 1987; Johannes & Riepen 1995; Wood 2001). Both the breaking of coral and the use of bleach to collect aquarium fish have been observed in Hawaii, although they are prohibited by law (W. Walsh, personal communication). We examined differences in coral cover and the incidence of broken and bleached coral as indicators of these effects. Although some differences were noted in the extent of bleaching and coral cover among study areas, there were neither consistent nor significant differences between control and effect sites that would indicate the presence of destructive fishing practices.

An issue of more general interest is the extent to which large-scale removal of herbivorous fishes can alter reef community structure. Four of the aquarium fishes (*A. achilles, C. potteri, N. lituratus, Z. flavescens*) accounted for 61% of the herbivorous fishes at the Honokohau and Red Hill control sites. These species were reduced in overall mean abundance by 32% at the effect sites relative to the control sites. Given that herbivorous grazers control algal populations that can overgrow corals (review by Hixon 1997), it is of interest to examine the community structure in areas where herbivory is reduced. Macroalgae were rare at all study sites, suggesting that reductions in herbivory associated with aquarium-fish collecting did not have a significant effect on this group of algae. However, our study may not be a good test of this hypothesis for several reasons. First, based on the model of Littler and Littler (1984), algae may be limited more by nutrients than herbivores. Second, with the exception of *N. lituratus*, the herbivorous aquarium fishes fed primarily on filamentous algae, not macroalgae. Filamentous algae are not easily surveyed by our photographic methods, so we collected no data on their abundance. Lastly, other reef herbivores, such as sea urchins, may control macroalgal populations, so reductions due to aquarium collecting may not be functionally significant. Given the global scope of aquarium harvesting on coral reefs, this question warrants further investigation.

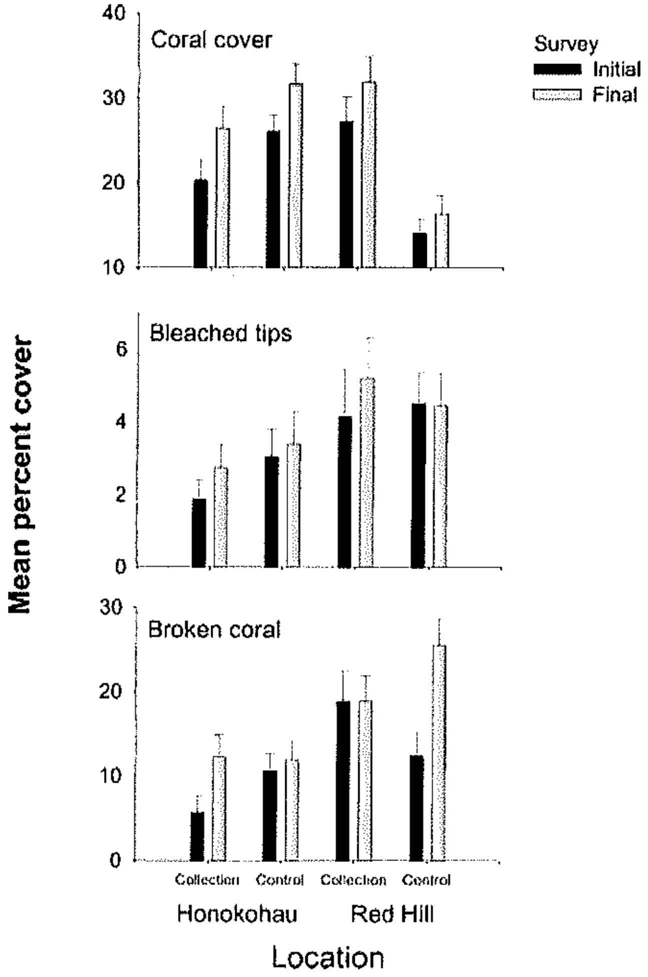

Figure 5. Changes in the mean percent (±1 SE) coral cover, bleached coral, and percent broken coral at control and collection sites in each study area at the beginning and end of the study.

Implications for Fishery Management

Aquarium collectors had significant effects on 7 of the 10 species of reef fish we examined. To determine whether these abundance patterns were clearly due to aquarium fish collecting will require better knowledge of the intensity and location of collecting activities. Although there are currently about 50 permits issued to collectors in western Hawaii, the number of active collectors is likely to be lower (W. Walsh, personal communication). The current system of catch reporting in Hawaii is limited to monthly collecting reports, with the 235-km coastline of western Hawaii divided into three large sections (Miyasaka 1997). Moreover, because these reports are not compared with actual catches, there is no assurance that the reports are accurate. Analysis of the current catch reports indicates that a significant portion of the monthly reports are not filed, although collectors are required to file a report even if no fish are collected (W. Walsh, personal communication). More specific information about location, catch, and effort are essential to support the results of this study. Moreover, random monitoring of collectors' catch reports would provide some level of quality assurance for these data.

We focused on major targeted species and did not collect data on rare species. Of the 103 species collected statewide, many are considered uncommon or rare and could also be threatened by overexploitation. For example, based on 1994-1995 collection reports, 204 Tinker's butterflyfish (*Chaetodon tinkeri*), a rare, deep-water species, were collected in western Hawaii and may possibly be overcollected. Other rare aquarium species, such as the Hawaiian turkeyfish (*Pterois sphex*) and the flame angelfish (*Centropyge loricula*), are also of concern and should be considered in future monitoring and management plans.

The magnitude and extent of the effects we documented and their relationship to the sustainability of aquarium collecting are problematic but warrant further investigation. In response to continued public outcry over the collection of aquarium fish, the Hawaii state legislature passed a bill in 1998 that focused on improving management of reef resources by establishing the West Hawaii Regional Fishery Management Area. A major component of the bill is to improve management of the aquarium industry by declaring a minimum of 30% of the western Hawaii coastline as fish replenishment areas (FRAs), protected areas where aquarium-fish collecting is prohibited. Based largely on input from the West Hawaii Fisheries Council, a community-based group of individuals, a network of nine FRAs was established in January 2000 as part of a plan to manage the aquarium industry. Current efforts are focused on monitoring these areas to evaluate the effectiveness of the reserve network as a fishery management tool.

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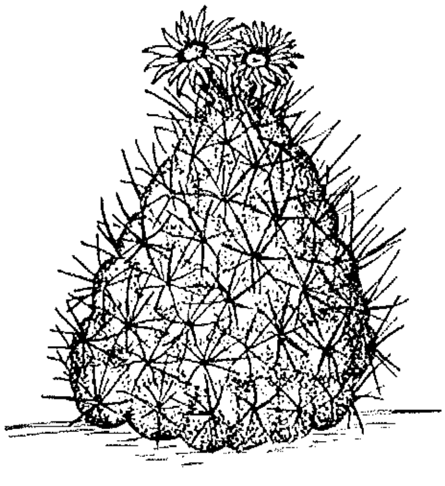

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Advances in Breeding and Rearing Marine Ornamentals

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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, overfishing, 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; Alcala 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-μm length) to copepodids III (CIII; 560-μm length) and used for feeding studies. Nauplii and copepodites were attracted into a 155- to 300-μm 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 μm 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 semicircle angelfish, *Pomacanthus semicirculatus*. Different diets were tested on larval survival rate and a diet composed of 30% *Gonyaulax* sp. (dynoflagellate) + 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 (Kuiter 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 interclutch 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 nonliving 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 "cleanup 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 $Mg(OH)_2$ 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 μmol/m^2 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 *Sinularia 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 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 Fernandéz 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-μm 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-μm 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 crowbars) 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. sulptus*, 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). Martinez 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 Tlusty (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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Growth and survival of porkfish (*Anisotremus virginicus*) larvae: comparing rotifers and copepod nauplii during first feeding

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Abstract. Eggs of porkfish (*Anisotremus virginicus*) were obtained from natural spawning events occuring at Seaworld Orlando and transported to the Tropical Aquaculture Laboratory. Eggs were stocked into 12 L experimental rearing tanks at 30/L to determine growth and survival of larvae fed rotifers (*Brachionus plicatilis* - Cayman strain) and copepod nauplii (*Acartia tonsa*) during first exogenous feeding through seven days post hatch (dph). Larvae fed rotifers during first feeding exhibited higher survival than those fed copepod nauplii (35.7 ± 4.0% and 23.8 ± 4.0% respectively). Conversely, larvae fed copepod nauplii exhibited greater body length and body depth than those fed rotifers. Results suggest that porkfish is an ideal candidate species for existing aquaculture technologies. This study highlights the Rising Tide Conservation Initiative, a collabortion of researchers and stakeholders invested in expanding marine ornamental aquaculture and research.

Key Words: *Anisotremus virginicus*, porkfish, larval rearing, copepods, rotifers.

Introduction. The marine ornamental fish trade relies primarily on wild-caught specimens to supply both private and public aquariums (Wabnitz et al 2003). Currently, aquaculture protocols are available for roughly 80 of the over 1,800 species of marine fishes traded in the international aquarium industry (Rhyne et al 2012; Wittenrich 2007). Aquaculture success with marine aquarium fish has typically been limited to demersal spawning species exhibiting parental care during incubation, low fecundity, and large larval size (Holt 2003; Olivotto et al 2011). Success with pelagic spawning species has been limited (Moe 1997, 2003; Ogawa & Brown 2001). As marine ornamental aquaculture moves progressively forward, developing production protocols for pelagic spawning species is becoming increasingly important (Sadovy et al 2001; Sadovy & Vincent 2002).

Bottlenecks to the commercial production of marine ornamental fishes have been generalized to include: initiating reproduction in captivity, development of eggs and larvae to the first feeding stage, and transition to first exogenous feeding (Olivotto et al 2006). Much research emphasis has been placed on the first feeding stage since successful feeding and subsequent growth of larvae determines the success or failure of aquaculture ventures (e.g. Moorhead & Zeng 2010; Wittenrich et al 2007; Olivotto et al 2006). Copepods have remained at the center of this research due their prevelance in the guts of wild collected larvae and high lipid content (Sampey et al 2007). The widespread use of copepods, however, has been limited due to captive production bottlenecks (Moorhead & Zeng 2010). Studies have demonstrated increased growth and survival of larvae fed copepod nauplii through the larval stages (Olivotto et al 2006, 2008), but it is still poorly known if limited or stage-specific feeding of copepod nauplii produce significant improvements to larval rearing.

The University of Florida's Tropical Aquaculture Laboratory (TAL) and SeaWorld Orlando (SWOR) are participants of the Rising Tide Conservation Initiative (RTCI).

Initiated by SeaWorld Parks and Entertainment in 2009, RTCI is a collection of research facilities, industry partners, and Association of Zoos and Aquariums (AZA) institutions committed to establishing viable aquaculture strategies for the commercial production of marine ornamental fish species (http://www.seaworld.org/rising-tide/index.htm). One of RTCI's initial research efforts utilize established populations of marine fish species spawning in public aquaria. Display aquariums are highly advantageous for research efforts focused on developing new species technology. Efforts of RTCI stakeholders take advantage of this valuable resource by collecting newly spawned eggs and/or larvae and shipping them to TAL for subsequent larval rearing studies; alleviating the necessity of on-site broodstock maintenance while providing screening of potential commercial species.

Recently, TAL and SWOR developed reliable techniques for harvesting and transporting the pelagic eggs of porkfish (*Anisotremus virginicus*) which lead to the establishment of larval rearing techniques for the species. Porkfish, a widespread Atlantic haemulid (Hoese & Moore 1998), are popular display animals in public aquariums due to their schooling behavior and bright colors. Similarly, juveniles of the species are captured for the aquarium trade where they display cleaning behavior (Brockmann & Hailman 1976). In the present study, pelagic eggs from SWOR were transported to TAL to determine the effects of first feeding larval diets on growth and survival. While porkfish have been reared in captivity prior to this study (Potthoff et al 1984) little information on the early life history and aquaculture potential is available. The main goal of this study was thus, to determine the relative growth and survival of larvae fed rotifers and copepod nauplii during the critical period of first feeding in hopes of contributing to an aquaculture protocol for the species and those closely related.

Material and Method

Spawning, collection, and egg incubation. Marine fish eggs were collected from the floor aquarium located in the Jewels of the Sea exhibit building at SeaWorld in Orlando, FL. The octagonal shaped exhibit is 6 m wide and 2.1 m deep containing approximately 106,000 L of artificial saltwater. The life support system contains four 3-foot sand filters (Triton T-140), a protein skimmer (Emperor Aquatics), heat exchanger and an in-house constructed nitrogen-reduction chamber. Water flows from the exhibit through a bottom drain and side skimmer to a sump. Four circulation pumps (1.5 HP Jacuzzi pump) draw water from the sump manifold to the sand filters before returning it to the exhibit. Water is pumped through side loops (0.75HP Jacuzzi) that split to a protein skimmer and heat exchanger before returning to the sump. The exhibit contains 12 species of tropical marine fish. Fish are fed approximately 2,000 g of food (capelin, mackerel, silversides, krill, squid and shrimp) two times daily.

Eggs were collected from the side skimmer using aquarium nets (500 µm mesh) that were reshaped to fit inside the skimmer box. At 3 pm, the nets were set in place and remained overnight. At 7 am the following morning eggs were transferred to a 1 L plastic container where infertile or dead eggs were allowed to settle. Viable embryos were then transferred to an 18.9 L bucket and transported to TAL. The bucket was temperature acclimated for 1 hour before carefully decanting viable eggs into an acclimation vessel composed of equal parts transport water and larval rearing system water. After one hour the eggs were gently homogenized before determining the number of eggs. Species composition was determined by differences in egg diameter and pigmentation

Larval rearing. Larvae were reared in 12 L cylindrical, flat-bottom fiberglass tanks connected to a recirculating filtration system. Tank bottoms were painted white to facilitate behavioral observations and prey densities and tank walls were painted black. Natural filtered and sterilized seawater was circulated through mechanical filter (40 micron filter sock), biological filter (Kaldness beads), protein skimmer (Precision Marine) and 15 watt UV sterilizer (Aqua). Photoperiod was maintained at 24L:0D throughout the experiment with two 30 watt fluorescent lamps (6500K) suspended 20 cm above the surface of the water. Eggs were stocked at a density of 30 eggs/l yielding 360 eggs per tank. Oxolinic acid was used at 1mg/L in the system water on days 1-10 post hatch. The initial water exchange rate was 400% total daily tank volume (TDTV) accompanied by gentle aeration. Beginning on 1 DPH, tanks were inoculated daily with Tahitian strain *Isochrysis galbana* (T-ISO). At 5 DPH, aeration was increased slightly and water exchange rate increased to 600% TDTV. At 10 DPH, aeration was again increased slightly and the water exchange rate increased to 800% TDTV for the remainder of the trial. Internal standpipes were fitted with 150 µm screen. Temperature was maintained at 28.3 ± 0.3°C, salinity 32.9 ± 0.8 g L^{-1}, pH 8.4 and dissolved oxygen 6.0 mg L^{-1}. Water quality via total ammonia-nitrogen was measured twice weekly with a Hach DR/4000U spectrophotometer. When TAN exceeded 0.015 ppm a 20% water change was performed on the total system volume via water exchange from the sump.

Live feeds culture. Rotifers, *Brachinous plicatilis*, Cayman strain (180µm lorica length), were cultured at 26°C and a salinity of 25ppt in four 110 L rectangular glass aquaria. Cultures were fed 1-4 L live *Nannochloropsis oculata* and Tahitian strain *Isochrysis galbana* (T-ISO) daily depending on rotifer density. Density of cultures was maintained below 500 mL^{-1}. *Acartia tonsa*, a calanoid copepod, were batch cultured at 28-30°C and a salinity of 22-25 ppt in four 150 L square polyethylene tanks and a 400 L cylindrical, conical-bottom tank with moderate aeration. Each 150 L culture was fed 0.5 L of T-ISO (15-20 million cells mL^{-1}) and the 400 L culture was fed 1.25 L. Nauplii were harvested twice daily from each tank via floating airlifts (Cassiano 2009). Harvested nauplii were then placed in a graduated beaker and quantified prior to feeding.

Experimental design. Two diet treatments were tested during first feeding (2-7 dph) to determine the relative growth and survival of larvae fed different diets during the transition to exogenous feeding. In group R, rotifers were maintained at a density of 2.0 mL^{-1} on 2 dph, 5.0 mL^{-1} from 3-5 dph, and 8.0 mL^{-1} on 6 dph. Larvae in group AT received *A. tonsa* nauplii at a density of 1.0 mL^{-1} on 2 dph, 2.5 mL^{-1} on 3 dph, 3.0 mL^{-1} on 4 dph, 4.5 mL^{-1} on 5-6 dph.

Diet treatments were limited to the first feeding stage, defined as the period of reduced feeding performance, prior to development of the opercular-linkage (Wittenrich & Turingan 2011). On 7 dph both treatments were fed rotifers at 10.0 mL^{-1} and increased to 15 mL^{-1} from 8-12 dph. Similarly, both treatments were fed *Artemia* nauplii at 0.5 mL^{-1} on 10 and 11 dph, 1.0 mL^{-1} on 12 and 13 dph, and 2.0 mL^{-1} on 14-15 dph. Live prey density was maintained by two daily feedings.

At 15 dph, all larvae were harvested and over dosed with MS-222 (tricaine methanosulfonate) prior to fixation in 10% buffered formalin. Specimens were transferred to 70% alcohol after two days. Transition to *Artemia*/dry feed greatly reduced mortality in previous trials at 15 dph and was used as the harvest date for the present study.

Sample collection and morphometric analysis. Morphometric analysis of fish larvae was adapted from Cassiano et al (2010). Twenty larvae from each experimental tank were haphazardly sub-sampled for morphometric analysis. Preserved larvae were placed on a sedgewick rafter cell, and photographed using a dissecting microscope at 40-X magnification with a digital camera (Sony Model DCRA-C171). SigmaScan Pro 5.0 image analysis software (SPSS Science) was used to measure: standard (SL) and body depth (BD). When measuring yolk-sac larvae, BD was considered the distance perpendicular to the longitudinal axis from the dorsal crest through the midpoint of the yolk-sac to the ventral most point of the body.

Statistical analysis. All statistical analyses were performed with SAS version 8.02 software (Cary, NC). Percentage data were arc-sine-square-root transformed prior to analysis. A Student's t test, following the TTEST procedure of SAS, was used to detect differences in treatment means for all dependent variables. All statistical tests were considered significant when $P \leq 0.05$.

Results and Discussion

Two species of marine fish eggs were identified in the SWOR collection. Porkfish eggs were dominant at 70.4% and blue striped grunt, *Haemulon sciurus*, constituted 29.6% of the samples. Very few larvae of *H. sciurus* survived beyond 5 dph (23 total among 12 replicate tanks), presumably due to interspecific competition from porkfish larvae. Growth and survival was not analyzed due to low numbers and competitive interactions.

Growth. Newly hatched porkfish larvae (1 dph) measured 3.52 ± 0.21 mm SL (mean ± SD) and 1.16 ± 0.09 mm BD. At 15 dph, The SL of larvae fed the AT diet was significantly greater than larvae fed the R diet (7.07 ± 0.96 mm vs 6.33 ± 0.84 mm; T198 = 5.73; $P < 0.0001$) (Table 1). The BD of larvae fed the AT diet was significantly greater than larvae fed the R diet (2.14 ± 0.30 mm vs 1.95 ± 0.24 mm; T198 = 4.94; $P < 0.0001$).

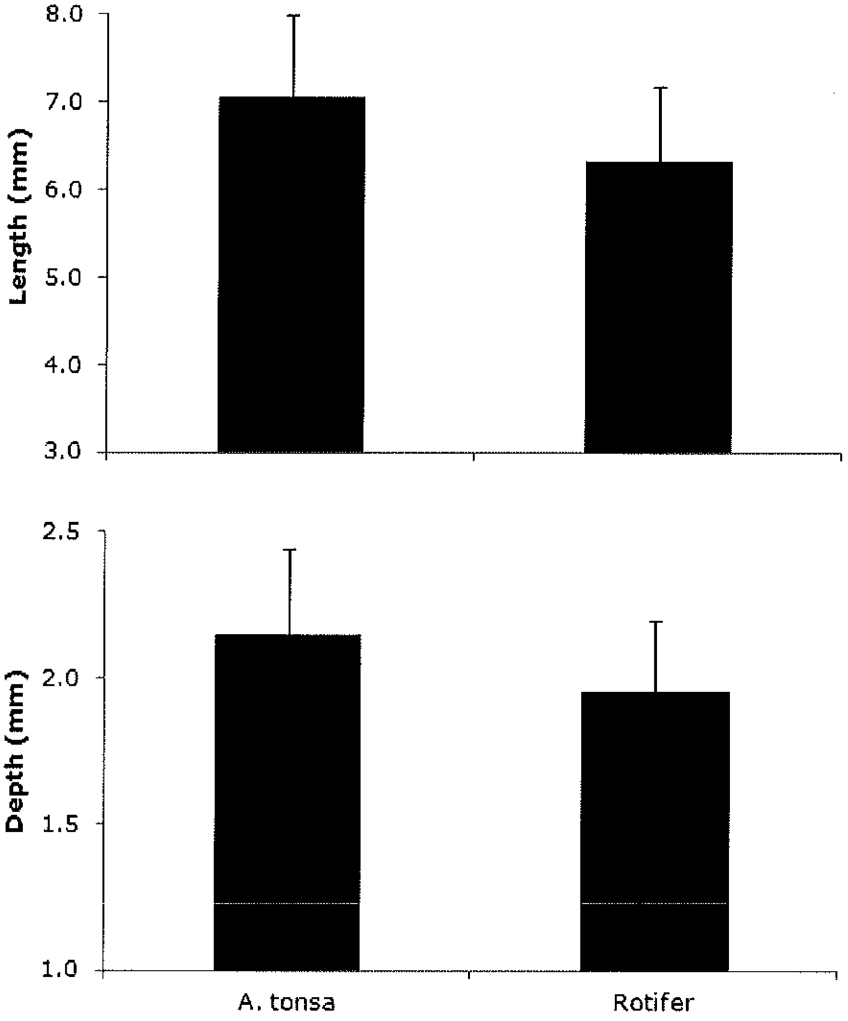

Figure 1. The standard length (top) and body depth (bottom) of 15 days post hatch porkfish larvae fed different dietary treatments during the experimental trial. Error bars represent standard deviation.

Survival. The survival of all larvae fed the R diet (35.7 ± 4.0%) was significantly greater than larvae fed the AT diet (23.8 ± 4.0%) (T8 = 4.64; $P = 0.0017$). The survival of porkfish larvae fed the R diet (50.2 ± 5.4%) was significantly greater than porkfish larvae fed the AT diet (33.1 ± 4.9%) (T8 = 5.20; P = 0.0008) (Figure 2).

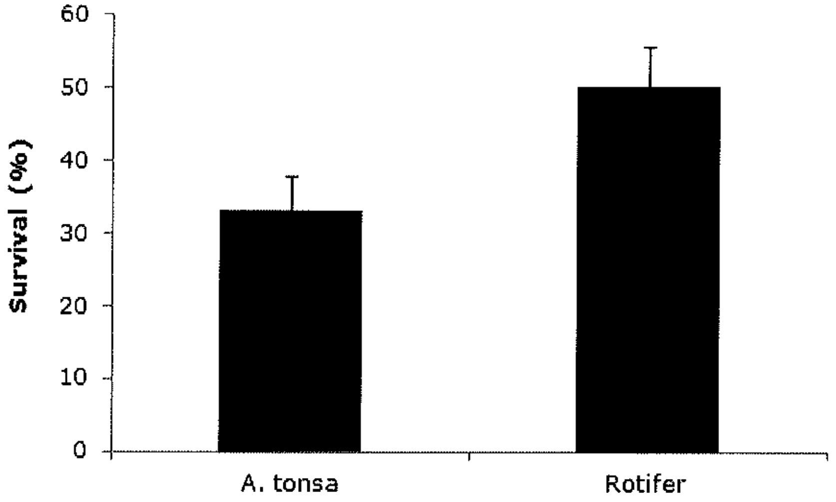

Figure 2. The percent survival of 15 days post hatch porkfish larvae fed different dietary treatments during the experimental trial. Error bars represent standard deviation.

Perhaps the most unique attribute of this examination was the utilization of marine fish eggs spawned in a public aquarium. As most production bottlenecks occur during the larviculture phase, continuous access to multiple species of marine fish eggs streamlines research efforts. RTCI has initiated a project using this valuable resource as a screening tool for the discovery of candidate species for marine fish aquaculture. With numerous species yet to be explored, examination of species readily spawning in public aquaria is viewed as a priority of research. This not only allows for multiple species to be examined simultaneously but reduces the need to acquire, acclimate, and condition broodstock. The porkfish is viewed as a successful model resulting from this approach with hopes that other species can follow suit. While the commercial value of porkfish within the aquarium hobby is limited, there is a sporadic, but significant demand for large numbers in public displays which may prove to be profitable for one or two producers.

Large-scale production of marine aquarium fishes is generally limited by the production of live feeds available during the transition to exogenous feeding. Many target species have not been successfully raised using traditional aquaculture prey items such as *Brachionus* spp. and *Artemia* spp. and there is continued concern that these prey types are nutritionally inferior to wild type zooplankters (Holt 2003). As a result, copepods have been heavily studied in recent years as a natural, nutritious first food choice for cultured marine fish larvae (Olivotto et al 2006, 2008). The success of using copepod nauplii for marine fish rearing is dependant on the species of fish in culture and the copepod chosen as feed. Tremendous diversity of mouth gape, prey capture performance, preference and nutritional requirements have been observed across marine fish families and it is likely that not all copepod species are suited for the culture of marine fishes. A few studies have noted that larval performance is greater in treatments co-fed with copepods and rotifers (Cassiano et al 2011; Stottrup & Norsker 1997). In species that recognize rotifers as prey items this technique can be advantageous. Unfortunately, many species don't identify rotifers as a first feeding organism and therefore the use of other prey items must be investigated.

Interspecific interactions were likely to have occurred in this study. Although unavoidable here, it is unlikely that growth and survival of porkfish larvae were greatly affected since these larvae appeared to be the dominant species and seemed to have a competitive advantage in size, mouth gape and behavior over *H. sciurus*. It is unlikely that this interaction improved the performance of porkfish larvae during this trial, but it should be acknowledged. In subsequent larval rearing trials, with and without the presence of other species, porkfish have exhibited similar developmental and survival patterns as presented here (Moe personal communication).

In this study, porkfish larvae fed rotifers during first exogenous feeding exhibited higher survival compared to those fed copepod nauplii. The prey density of copepod nauplii was maintained lower than that of rotifers throughout the experiment. This is a common feeding strategy due to the higher nutritional content of copepod nauplii, however, the low prey density may have affected feeding performance of early larvae. The growth of larvae fed copepods, however, was greater than those fed rotifers. Improved growth in the copepod treatment could be explained by reduced larval density due to mortality, which then offered higher prey densities to surviving larvae. The acceptance of rotifers during first exogenous feeding, and the subsequent survival, suggests that copepods are not needed to implement large scale production of porkfish.

In Potthoff et al (1984), porkfish were reared, however, larvae were fed size sorted wild zooplankton with little details toward large scale culture. The present study shows that porkfish readily accept rotifers (*Brachionus plicatilis*) as a first food and are well suited to current commercial marine aquaculture technologies. Preliminary results at TAL encouraged a relationship between three industry partners and SWOR wherein eggs from SWOR were delivered to three commercial hatcheries for larviculture and growout. Two of the three facilities were successful in rearing large numbers of juveniles that were sold back to SWOR. This unique approach proved effective in obtaining viable numbers of eggs for examination. The model used here is viewed as a research priority for expanding marine ornamental species aquaculture.

Conclusions. The role of public aquariums to streamline marine fish aquaculture research is a great resource that should continue to be utilized. Based on the results of this study, porkfish have potential for commercial aquaculture as they fit the mold of current larviculture techniques (the primary bottleneck to production); specifically the live feed organism used. Futhermore, the practice of harvesting porkfish eggs from a public aquarium, growing them in a commercial facility, and selling them back to that public aquarium (and the retail market) has been demonstrated. This can be a model for all species in the quest for sustainability within public aquariums.

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A NEW DEVICE FOR COLLECTING DRIFTING SEMIBUOYANT FISH EGGS

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A New Device for Collecting Drifting Semibuoyant Fish Eggs

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Abstract.—Several fish species in lotic systems are pelagic broadcast spawners that produce nonadhesive, semibuoyant eggs that drift downstream. This reproductive strategy and egg type appear to be common in Plains stream cyprinids in the west-central United States. Although it is relatively easy to capture semibuoyant eggs, the inability to provide species-specific identification of this life stage has hindered studies on the reproductive ecology and life history of these fishes. While drift nets have been used to collect semibuoyant eggs, the process of separating the reproductive products from other organic drift was time consuming and usually fatal for eggs. We developed a field sampling device, the Moore egg collector, that allowed for the efficient, quantitative, and nondestructive collection of large numbers of semibuoyant fish eggs and that could aid in the study of a variety of organisms that employ drift as a dispersal strategy during a portion of their life history.

In the summer of 1940, George A. Moore and students (University of Oklahoma) initiated life history studies of Arkansas River drainage cyprinids in the Cimarron River in Oklahoma. The principal objective of their study was to obtain information on breeding habits and early developmental stages of two Plains stream fishes, the Arkansas River speckled chub *Macrhybopsis aestivalis tetranemus* and the Arkansas River shiner *Notropis girardi*. Moore (1944) reported the capture of numerous nonadhesive, semibuoyant fish eggs as one of his students held a wire mesh screen (1.6-mm mesh) in the waist-deep flow of the river. In order to determine the specific identification of the eggs, Moore (1944) reared the eggs through larval developmental stages and ultimately identified them as Arkansas River shiner.

Data obtained from Moore's reared-fish collections allowed for a better understanding of aspects of the life history of Plains stream fishes. From egg type morphology and larval fish development and behavior, Moore (1944) speculated that Arkansas River shiner spawning was stimulated by intense summer rainstorm events. He also noted the rapid hatching (24 h) of the relatively large eggs (ca. 2.5 mm in diameter) and the swim-up behavior of the larvae. Bottrell et al. (1964) discovered that Arkansas River speckled chub and plains minnow *Hybognathus placitus* also produced semibuoyant eggs and that the larval fish development and behavior of these two species was similar to that noted by Moore (1944) for Arkansas River shiner. Most recently, Platania and Altenbach (1998) reported this reproductive strategy and egg type in three additional Plains stream cyprinids: Rio Grande silvery minnow *Hybognathus amarus*, Rio Grande shiner *Notropis jemezanus*, and Pecos bluntnose shiner *Notropis simus pecosensis*.

In 1993, we used drift nets to collect and determine the catch rate of semibuoyant eggs from members of this reproductive guild (plains minnow, Arkansas River speckled chub, Arkansas River shiner, Rio Grande shiner, and Pecos bluntnose shiner) in the Pecos River in New Mexico. (In this paper, the term drift net refers to a 0.5-m–diameter mouth, 4-m–long, 560 μm–bar-mesh plankton net fitted on a 36 × 46–cm rectangular frame.) The spring and summer rainstorm events that triggered spawning by guild members also delivered substantial amounts of debris into the river. We observed that the majority of semibuoyant eggs collected during the 1993 study were damaged or killed either during the collecting or the sorting process.

Moore's serendipitous collection of eggs in 1940, made with a screen, provided the impetus for the techniques we developed, techniques that have resulted in more detailed studies of this cyprinid reproductive guild. This paper reports the design of a device that passively sorts and concentrates semibuoyant eggs from instream debris so that the undamaged eggs can be easily collected and reared to an identifiable larval stage. In recognition of the pioneering efforts of G. A. Moore in the study of the ecology of this reproductive

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guild of Plains stream fishes, we name this device the Moore egg collector (MEC).

Methods

Design and construction.—The Moore egg collector is a sluice-box–like device, with a rectangular opening at its upstream end (width = 45 cm; height = 33 cm), parallel wooden sides (length = 119 cm), and an open top (Figure 1). The bottom is framed fiberglass window screen (1.6-mm mesh) that is installed at a 23° angle relative to the bottom mounting bar. Mounting bars are attached near the posterior end of the mouth and perpendicular to the sides. This device is relatively inexpensive, requiring less than US$100 in materials, all of which are available at most hardware centers, and it can be built in 1 d.

The MEC is constructed of 9.5-mm–thick C : D-grade plywood, 25 × 51–mm pine furring strips, and zinc-plated wood screws, and it weighs about 6.8 kg. Aluminum screen framing (7.94 × 19.06 mm) and plastic screen corners are used to make the 43 × 104–cm filtering screen. Damaged screens can be easily replaced, and different mesh sizes can be used to suit individual study objectives. The screen frame is fastened with small wood screws to the parallel pine furring strips that are attached to the interior wall at the base of the MEC. The wedge-shaped water diverter is 90 mm tall at the edges, 130 mm high at the creased (150°) midpoint, and is cut from 2.4-mm aluminum plate. Angled (90°) mounting brackets, bolted with machine screws to both the back of the diverter and top of the flume, hold this piece in place.

The illustrated version of the sample holding cup comprises two relatively simple polyvinyl chloride (PVC) components (Figure 1) and can be easily modified by users to accommodate their needs. We attached a 50.8 mm–diameter PVC plug to the posterior cross-member (with wood screws) and cemented a 50.8 mm–diameter coupling to the plug. The sample holding cup is made of a 152.5-mm segment of schedule 40 PVC (50.8 mm diameter) pipe sealed at the bottom by a test cap and silicon and at the top by a pressure cap. The holding cup is held in place by the coupling sleeve. The upper and lower ends (exteriors) of the PVC pipe (=sample holding cup) are sanded so that neither the fit of the pressure cap nor its fit in the coupling is too tight.

Flowmeters can be easily attached by placing mounting holes in the walls of the mouth of the MEC or by attaching small pieces (ca. 4 × 4 cm) of aluminum plate, with a predrilled attachment hole that overhangs the mouth by about 2 cm, into the tops or sides (not illustrated). Flowmeters can be mounted top to bottom (as illustrated) or side to side. As with construction of the sample holding cup, individuals can modify the flowmeter attachment mechanism. In order to extend the life of the collecting device, all wood surfaces should receive multiple coats of high-quality marine varnish.

Field application.—During operation, the MEC is held in place by the force of the water that is pushing the device against metal fence posts (t-posts) that are driven into the stream bottom (Figure 2). Electric fence insulating brackets are fastened, as per manufacture's instructions, at about the same level on each t-post. The brackets support the weight of the MEC and allow for its vertical adjustment. Operating the device just below the water's surface prevents collection of floating debris and allows the aluminum water diverter, positioned on top, to reduce drag under high-velocity conditions. The posterior t-post and electric fence insulating bracket support the downstream end of the collecting device. Drift that enters the MEC becomes impinged on the screen, while the current pushes the spherical, semibuoyant fish eggs up the inclined screen. Eggs accumulate at the water–air–screen interface, where they can be easily gathered, counted, and collected with an eyedropper before being placed in the water-filled (and removable) sample holding cup.

Particulate matter that accumulates on the screen and impedes the flow of water can easily be removed either by hand or by immersing the downstream end of the MEC into the water. The interim between screen cleaning is dependent on the amount of debris in suspension and on the velocity of water being sampled, but in our experience, this interim ranged between 30 s and several hours. Capture rate of drift by the MEC should be determined based on the volume of water (i.e., catch per unit effort [CPUE]) filtered as opposed to sampling duration. This is accomplished by mounting a flowmeter in the mouth of the MEC and by calculating CPUE using the area of the mouth and the appropriate formula for the flowmeter employed.

Results and Discussion

Comparison of Drift Net and MEC Catch Rates

A comparative investigation of drift net and MEC egg-catch rates was performed on nine oc-

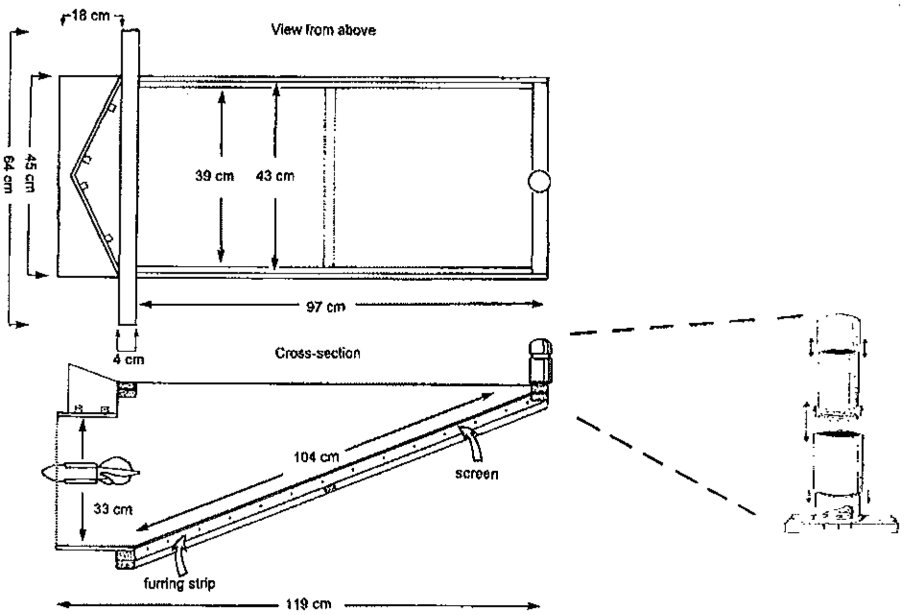

FIGURE 1.—Schematic of the Moore egg collector showing top and horizontal or side views.

casions in the Pecos River (New Mexico) during the period of 9–14 June 1997. The two devices were set simultaneously in similar velocity habitats and within 1 m of each other. About 19,000 eggs were taken in the 18 samples of this preliminary study. More than four times as many eggs were collected in the MEC compared with the drift net, despite the fact that we sampled similar volumes of water. The amount of time that the two devices were operated was not comparable, as the drift net was fished almost twice as long as the MEC in order to sample the equivalent volume of water. A paired two-sample *t* test of this data set demonstrated significant differences ($P = 0.0090$) in CPUE between the drift net and MEC (Table 1).

Frequent screen cleaning of the MEC improved the sampling efficiency of the device and resulted in larger collections of semibuoyant eggs than were obtained with drift nets, even though similar volumes of water were sampled. As debris accumulated in the drift net, filtering efficiency decreased, likely in response to increased resistance to flow through the net. The number of eggs collected in a given volume of water began to decrease once there was a noticeable resistance to flow through the drift net, and this decrease became marked when instream debris levels were high. A potential explanation for the differences in catch rate between devices was that the natural hydrologic path followed by drifting fish eggs approaching the drift net was altered because that device accumulated debris. This ultimately resulted in a situation in which fewer semibuoyant fish eggs entered the drift net.

Conclusion

The MEC is more cost effective, efficient, and informative (=quantitative) than fine-mesh seines or drift nets in terms of the collection of drifting semibuoyant fish eggs. Two people are required to operate a seine, and any eggs collected are difficult to locate and remove. Since the volume of flowing water sampled during seining cannot be quantified, an accurate catch rate cannot be determined. Conversely, while catch in a drift net can be quantified, the eggs are collected with large amounts of debris. The process of separating eggs from unwanted drift is time consuming and costly, and ichthyoplankton collected in the drift net are usually damaged or killed, either during the set or removal of the net. Continual cleaning of a drift net would likely improve its filtering efficiency and result in increased capture rates of semibuoyant eggs, but this would not be practical. Although it is generally easy to capture semibuoyant fish eggs using these two sampling methods (seines and drift nets), the inability of these methods to provide both accurate and quantitative catch rates and live specimens for subsequent

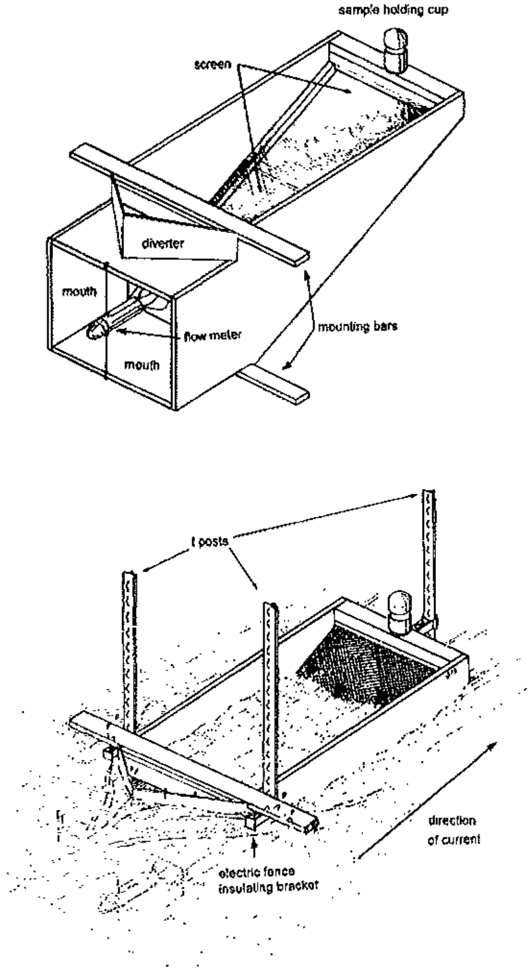

FIGURE 2.—Three-dimensional view of the Moore egg collector and a view with the collector in place in the river. The large arrowheads in the lower drawing indicate the area of semibuoyant egg accumulation.

TABLE 1.—Comparison between drift net and Moore egg collector (MEC) catch rates of semibuoyant eggs.

Measurement	Drift net	MEC
CPUE[a] for sample number:		
1	32.68	84.98
2	3.53	17.50
3	3.72	29.90
4	1.19	5.75
5	1.25	10.79
6	1.72	8.00
7	0.86	6.53
8	0.93	12.40
9	0.60	7.41
Number of semibuoyant eggs collected	3,416	15,580
Total volume (m^3) of water sampled	1,090	1,051

[a] Catch per unit effort (CPUE) was calculated per cubic meter of water sampled; sample times are not comparable.

species-specific identification of early life stages limits the utility of these sampling techniques in determining specifics of the reproductive ecology and life history of these fishes.

The MEC is designed for the rapid, quantitative, and nondestructive collection of large numbers of drifting semibuoyant eggs. It is a relatively inexpensive item that requires only one person to operate, and it can be employed effectively in both high- and low-velocity conditions. Most investigations will require that an individual remain with the MEC throughout the sampling period (i.e., 1–2 h). However, we found that when instream debris levels were low, the MEC could be left unattended for several hours, and it would still remain an efficient collecting device.

Although we designed and used this device to study the periodicity and magnitude of spawning by Plains stream cyprinids that produce semibuoyant eggs, its utility may benefit other ichthyological and ecological studies. There are numerous fish taxa other than Plains stream cyprinids that produce eggs that remain in suspension in the water column while drifting (e.g., Hiodontidae, Clupeidae, grass carp *Ctenopharyngodon idella*, striped bass *Morone saxatilis*, and freshwater drum *Aplodinotus grunniens*). The MEC also collects drifting larval fishes and aquatic invertebrates and may be applicable for studies that investigate the timing, duration, and magnitude of their drift.

We are currently experimenting with several design modifications of the MEC. Changes in screen type and configuration are being tested in an attempt to increase the ease of collection of larval fishes; we continue to refine the overall structure of the MEC with the goal of providing a more automated sampling device. Detailed quantitative laboratory and field studies designed to assess and explain differences in semibuoyant egg-capture rates related to drift nets and the MEC are ongoing.

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ENVIRONMENTAL AND ECOLOGICAL EFFECTS OF TRADE IN ORNAMENTAL CORAL REEF SPECIES

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The trade in ornamental species from coral reefs is a major international industry that targets over 1400 species of reef fishes, live rock, stony corals, gorgonians and molluscs, as well as many other invertebrates for aquariums, curios and jewelry. Recent estimates of the trade indicate it involves about 30 million fish, 1.5 million live corals, and over 1,000 tons of shells per year from over 45 countries primarily originating from the Philippines and Indonesia, with lesser amounts from Brazil, Maldives, Vietnam, Sri Lanka, Solomon Islands, Fiji, Red Sea and Australia. The US is the single largest importer of the trade and also exports fish from Hawaii, Florida and Puerto Rico.

Although there is a large volume of literature and data regarding the extent of trade impacts on coral reef ecosystems, there are very few rigorous studies, and thus the true impacts of the trade have a high level of uncertainty. To date, field studies performed have focused mostly on qualitative or quantitative observations of target species in collection areas; however, few studies have used uncollected control sites for comparison and many lack sufficient time-series to infer statistical significance. Although in recent years there has been increased efforts to develop and implement more rigorous studies in the Pacific (e.g., MACTRAC), there are relatively few that document statistically significant effects of the ornamental trade on coral reefs. Thus, the approach we have taken is to focus on well designed studies but also to include the large number of additional studies and observations to examine the range of uncertainty and to consider the cumulative and potentially cascading effects of impacts as a whole.

Because the trade is highly selective and often targets large quantities of species of value, the potential for overexploitation and thus for significant ecological effects on coral reef ecosystems is high. At particular risk are species of high value, short geographic ranges, rare and endemic species, species that experience irregular recruitment or have unusual breeding and rearing patterns (e.g., mouth brooding, monogamous mating), species in symbiotic relationships with target species, and those that are easily accessible. Thus, significant effects have been documented for seahorses, anemonefish and their associated anemones, the short-range endemic Banggai cardinalfish (*Pterapogon kauderni*), as well as live corals, molluscan shells and species targeted for curios such as sharks (for teeth) and dried seahorses as well as many other species. Harvesting of live coral is particularly destructive because it can cause localized destruction of coral reefs, including increased erosion and loss of critical habitat. Among the common reef fishes, damselfish are the most harvested, followed by angelfish, butterflyfish, wrasses, blennies, surgeonfish and another 15 or so families. Several well documented studies indicate that as much as 90% of these species may be removed from reefs by collector activities in some areas. In other areas, especially where the number of collectors is small or fish are infrequently harvested, there may be minor or non-significant reductions in abundance. Targeted reef fishes are typically juveniles and thus overharvesting can cause significant growth overfishing and reduce the population size of breeding individuals. Overharvesting of one sex of a species, which may occur in wrasses, anemonefish, and parrotfish, can cause significant Allee effects and further reductions in reproductive output.

Harvesting pressure is increased by post-collection mortality due to collecting, handling, holding and shipping which can range between 5-90% , thus requiring additional specimens to offset losses. This secondary mortality can occur due to the use of chemicals for capture, barotrauma, injury during capture and handling, disease, poor stocking practices, and poor water quality. In addition, by-catch associated with harvesting can result in mortality of non-target species. Thus, the reported number of exports is likely to be underreporting the actual number harvested in most situations, perhaps significantly in some cases, and the effects on coral reefs higher than deduced using export data.

In addition to the direct effects of harvesting target species, there have been numerous documented incidences of destructive practices associated with fish capture. These practices include the uses of nets, traps, poisons and explosives to capture fish. Even careful collectors using nets often snag, break or crush corals and thus can cause low but chronic reef damage which can cumulatively cause significant effects. In the Philippines and Indonesia aquarium collectors may also target fish for the live reef fish trade, which is associated with widespread and destructive collecting practices. The use of cyanide to stun and catch fish in the live reef and ornamental trade is widespread in 15 countries and can cause high mortality in targeted fishes as well as non-target fish, corals and other reef organisms. Collection methods used in conjunction with cyanide often cause significant impacts when captured organisms are removed from the reef. Although there have been efforts to reduce the use of cyanide in the some areas by establishing testing facilities, a 2008 report indicated 49% of live fish caught in the Philippines tested positive for cyanide.

Another significant secondary effect of the marine ornamental trade is the introduction of nonindigenous marine species, which represents a significant threat to biodiversity. Although ballast-waters from seafaring vessels have been implicated in dispersing marine organisms, recent studies suggest that the aquarium trade is also culpable and is an effective vector for disseminating invasive species and associated diseases. The more common pathways for aquarium species introductions include dumping of unwanted organisms, escaping from holding facilities, and draining/exchanging tank water. Aquarists occasionally release larger specimens into the wild due to the organism outgrowing its tank, and because these specimens are typically older, they are often reproductively viable. The taxonomic diversity associated with the aquarium industry increases the chance for a species to become invasive; however, studies show that the distance from where many of the aquarium species are sold in relation to local waterways, the treatment of discharged tank water, the tropical origin of most species, and increased awareness among store owners and aquarists help mitigate the risks of new invasions. The trade in live rock also poses serious concern because it involves selling whole communities of invertebrates as a collective unit, and many of these invertebrates are easily dislodged and introduced into the wild when aquarium tank water is exchanged.

Recent lionfish (*Pterois* sp.) invasions, which are likely to have been introduced from aquariums, have been documented in parts of the Caribbean and the Mid-Atlantic coast, and are showing large and significant effects on the native fauna as well as being dangerous to humans. Similarly, *Caulerpa taxifolia*, a common alga found in many aquariums, has spread across the Mediterranean, Australia, and the US, where it has displaced native species. It is impossible to predict the temporal or spatial occurrence of introductions and it is exceptionally difficult to control marine invasive species once they are established.

The cumulative impacts of direct harvesting, destructive fishing practices and introduction of invasive species and diseases can have strong negative effects on coral reef ecosystems and reduce a reef's resilience to respond to other anthropogenic impacts, such as climate change. Thus, the aquarium trade can affect more than just the size of populations; it can alter the entire dynamics of a reef. Many targeted fishes and invertebrates are herbivorous, and intense removal of herbivorous species can lead to reduced rates of herbivory, and when combined with land-based pollution can cause ecological phase shifts from coral to algal dominance and increase the incidence of coral diseases and coral bleaching. Other important functional groups that contribute to reef resilience include corals, species whose larvae move between habitats and increase connectivity, and predators such as sharks, and corallivores, such as many butterflyfishes. The functional diversity and interactions between these groups can be significantly affected by the multiple threats from the ornamental trade. Consequently, coral reef ecosystem resilience can be reduced, resulting in even greater impacts of subsequent disturbances such as coral bleaching from climate change and ocean acidification.

Although many countries with marine ornamental fisheries have some regulations regarding the trade, these tend to be limited and focused on specific issues, such as cyanide use, live coral/rock collection, and or limits on specific organisms (e.g., giant clams). However, very few countries have management plans and those that do are often ineffective. Thus management per se is often non-existent or difficult to implement in source countries and often de facto open access and overharvesting can lead to widespread ecosystem degradation. The number of ornamental collectors and exporters varies widely by country, but generally they are geographically dispersed making it difficult to manage, particularly in Indonesia and the Philippines, where migrants will travel long distances in search of harvesting opportunities. Although many countries require some form of monitoring and reporting catches, the data are often suspect because there is no way to verify the accuracy of the information and export quotas are based on the number of organism that survive to be exported, not on the number harvested. Thus management is often based on inaccurate data. There is evidence that the industry is underreporting the level of captured species, perhaps by as much as 30-90%, and the rate of harvesting species from the reef may be significantly higher in some species and areas than previously thought.

Management strategies include a wide variety of approaches that vary in their level of effectiveness. Limited entry, quotas, size limits, gear restrictions, closed seasons, traditional property rights, and marine protected areas have been used. Other approaches may provide restrictions on rare species or those at risk of overexploitation. Risk may be determined using a number of criteria, including biological criteria (geographic ranges, abundance, endemism, symbiotic species and recruitment , breeding and rearing patterns,) as well as its economic and non-economic value, rate of exploitation, accessibility, protection status, and use by other extractive industries, such as food fisheries. Successful management strategies have largely utilized traditional property rights, marine protected areas, community-based management, and/or limited entries and enforceable quotas.

Recommendations:

1. There are few data on the basic biology, life history, and ecological interactions of most targeted species, although some information is available through well-studied species in some cases. This information is critical to developing risk assessments and sustainable fishery management plans.
2. More rigorous studies needed to be implemented that quantitatively assess impacts of the ornamental trade on source species populations, communities, and their links to coral reef ecosystem resilience and susceptibility to coral bleaching and disease.
3. Studies quantitatively documenting invasive aquarium species impacts are needed to assess the full extent of non-indigenous species effects on native ecosystems. International cooperation for dealing with existing and new marine species invasions via the aquarium trade need to be established
4. The development of sustainability in the ornamental trade will require major changes in the entire process of collection, handling and transport of individuals. Training and certification, such as that provided by the Marine Aquarium Council (MAC), can help promote a sustainable trade but require funding for monitoring, evaluation, capacity building, enforcement and training.
5. Management plans need to be developed that protect at-risk species, critical habitats, key species, important ecosystem services, and prevent overexploitation of others species through appropriate measures. Implementing these plans represents a major challenge as the majority of

source nations do not have the enforcement, resources nor capacity to fund effective programs.

6. Although aquaculture of marine species shows promise as an alternate or additional source of individuals for the ornamental trade, economic forces may favor live capture in many species and it is critical to define standards, best practices and provide enforcement, otherwise the industry may merely be creating another environmental problem.

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