

Species Profile: Clownfish

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Clownfish, or anemonefish, are iconic members of the coral reef community and common residents of home and public aquaria due to their striking appearance, generally low cost, small size, hardiness, passive temperament in a community environment, and amusing behavioral traits. The clown anemonefish or false percula clownfish (*Amphiprion ocellaris*) is the most prominent clownfish species in the marine ornamental trade, ranking in the top ten imported species into the U.S. by volume, with hundreds of thousands of individuals entering the country every year. Aquacultured clownfish represent a significant contribution to the volume of animals currently in the trade. Commercial clownfish production dates back to the 1970's, amidst widespread doubt of the feasibility of marine ornamental species ever being produced in large enough quantities for the consumer market. Martin A. Moe, Jr. was a pioneer in this field, offering the first captive bred clownfish through Aqualife Research Corporation in 1973. Almost 50 years later, 25 species of clownfish have been successfully cultured in captivity, with about half of those species readily available for purchase.

Description and taxonomy

Clownfish are members of the subfamily Amphiprioninae, which distinguish themselves from the rest of the Pomacentridae, or damselfish, family primarily because of their unique symbiotic relationship with anemones. The "classic" clownfish phenotype has a small compact body [maximum size roughly 4 inches (10 cm)], with exaggerated rounded fins and three thick white bars against a bright orange background. Currently, there are 30 recognized species of clownfish with 29 belonging to

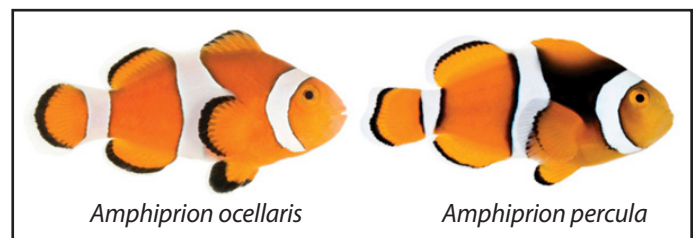


Figure 1. The clown anemonefish (*A. ocellaris*) can be distinguished from its close relative by counting the number of dorsal fin spines (11 in the clown anemonefish vs. 10 in the orange clownfish [*A. percula*]), observing the thickness of the black outline on the white bars (thinner in the clown anemonefish), and the height of the dorsal fin (taller in the clown anemonefish). Photos used with permission from Oceans Reefs and Aquariums (ORA) (Ft. Pierce, FL).

the *Amphiprion* genus and the final to the *Premnas* genus (Maroon clownfish). The clown anemonefish and the orange clownfish (*A. percula*) have a very similar appearance and are often mistaken for one another (Fig. 1). For simplification, only the two most prevalent clownfish species (clown anemonefish and orange clownfish) in the industry will be discussed and hereafter will be referred to simply as "clownfish" unless otherwise indicated.

Natural history

Range

Most clownfish species are found in warm shallow tropical seas in the Indo-West Pacific from the Red Sea to the Central Pacific with the highest abundance and diversity found in the Indo-Australian-Philippine region. The Clown anemonefish has a rather wide distribution and can be found in Northwest Australia, the East Indies, Melanesia, the Philippines, and surrounding the Okinawa islands. Orange clownfish have a more restricted range

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limited to Melanesia and Northeast Australia. Post-larval clownfish are largely sedentary and rarely travel more than a few meters from their host anemone, gathering all necessary resources within close proximity. Confinement to host anemones require clownfish to be omnivorous, opportunistic feeders, consuming various food items such as copepods, algae, worms, crustaceans, tunicates, and amphipods that reside within its territory.

Life history

Clownfish are protandrous hermaphrodites, meaning they are born male and have the ability to change sex to female depending on social structure. This is an important adaptation for the propagation of the species because clownfish are also monogamous and form single pairs of one, often older and larger dominant female and one, often younger and smaller submissive male. A clownfish pair will often inhabit one specific anemone along with a few younger individuals. If the female perishes, the male will transition to female over the course of 2 to 3 months and a young juvenile will take the vacant male position. Clownfish are demersal spawners, affixing adhesive eggs to a substrate, and will often position their nest at the base of a host anemone for protection. An active pair will generally spawn about once a month year-round. The eggs will incubate for about one week while the male guards and cleans the nest. Newly hatched, precocial larvae are relatively strong swimmers and can capture prey almost immediately. Clownfish larvae spend the first 2 to 3 weeks of their lives as pelagic larvae, occupying the upper level of the water column and feeding primarily on various types of zooplankton. The larvae undergo numerous morphological changes as they develop and transition into juveniles. This transition, commonly referred to as metamorphosis, is usually completed about two weeks post hatch and sexual maturity is normally reached in 9 to 15 months.

Clownfish – anemone symbiosis

The unique relationship between clownfish and anemones is classified as mutualistic, where both organisms benefit from the relationship. Clownfish reside within the tentacles of the anemone for protection from predators and the anemone receives food, in the form of uneaten and/or digested organic matter, from the clownfish. The nematocysts, or stinging cells, of the anemone do not harm the clownfish due to the thick protective mucus layer formed from repeated contact with the anemone. A specific anemone is thought to be chosen based upon location in relation to important environmental elements such as currents, light, reef habitat, and food availability.

In the home aquaria, clownfish commonly display this fascinating behavior and often inhabit other soft corals in addition to anemones.

Hybrids and color morphs

Clownfish which exhibit prototypical coloration and morphology are generally inexpensive, however, selective breeding over several generations has created “designer” clownfish resulting in body size, color, pattern, and finnage variations (Fig. 2) that cost considerably more (Fig. 3). Body



Figure 2. Longfin clown anemonefish morph first bred at Sustainable Aquatics (Jefferson City, TN). Photo used with permission from Segrest Farms (Gibsonton, FL).


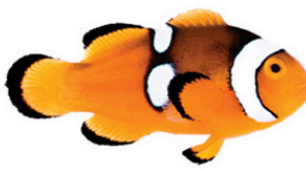


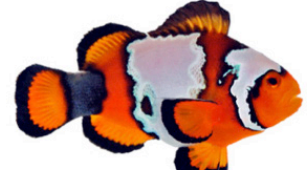

<i>Amphiprion ocellaris</i>	<i>Amphiprion percula</i>
 <p>"Black and White"</p>	 <p>"Misbar"</p>
 <p>"Naked"</p>	 <p>"Platinum"</p>
 <p>"Snowflake"</p>	 <p>"Picasso"</p>

Figure 3. The clown anemonefish and orange “designer” clownfish examples. Photos used with permission from Oceans Reefs and Aquariums (ORA) (Ft. Pierce, FL), FishEye Aquaculture (Dade City, FL), and Proaquatix (Vero Beach, FL).

patterns and colors that deviate from the wild phenotype are often referred to as “color morphs.” Hybridization among clownfish species is relatively common in the wild and is often deliberately orchestrated in captivity. Intraspecific hybridization may be used in directed breeding efforts to cross two individuals with specific phenotypes to produce offspring with desirable color morphs or create novel ones. Interspecific hybridization is an additional production strategy commonly used where reproductive crosses may include individuals of different species, one species and a hybrid, or two hybrids. The most common hybrid cross is a clown anemonefish with an orange clownfish, however, many other crosses have been successful.

Culture techniques

Systems

Access to natural seawater will dictate the system design and engineering for clownfish production. Simple flow through systems may be employed should seawater be readily available; however this is usually not the case. In general, all aspects of clownfish culture, including broodstock holding, larval rearing, and growout (Fig. 4), are carried out in separate, closed recirculating systems. In this type of system, water exchange is limited, requiring various types of filtration to maintain appropriate water quality. System components should include mechanical and biological filtration to keep toxic nitrogenous wastes to a minimum (Francis-Floyd et al. 2009), and ultraviolet sterilization to reduce concentrations of bacteria, parasites, and other harmful organisms. Additional water parameters should be maintained within the following ranges: temperature 79 to 82°F (approximately 26 to 28°C), salinity 30 to 35 parts per thousand (g/L), and pH 8.0 to 8.3. Water flow rates should be high enough to maintain water quality standards and can vary greatly dependent upon stocking density, feeding frequency and quantity, capability of filtration components, and system



Figure 4. Clownfish growout system. Photo used with permission from FishEye Aquaculture (Dade City, FL).

size. Please see SRAC Publication No. 0451, *Recirculating Aquaculture Tank Production Systems: An Overview of Critical Considerations* and SRAC Publication No. 0452, *Recirculating Aquaculture Tank Production Systems: Management of Recirculating Systems* for more information regarding the design and management of recirculating systems.

Broodstock and spawning

A clownfish broodstock tank should be at least 10 gallons (~38 liters), although 20 gallon (~76 liters) high glass aquaria are often used for commercial production. Each broodstock tank should have a bare bottom, and three opaque sides to reduce stress by blocking external stimuli and reduce aggressive behavior between conspecifics in adjacent tanks. The only habitat that should be in the tank is the spawning structure/shelter, excess structure (including anemones) is not necessary. The most common structures used are terracotta flower pots, tile, and slate, although large diameter PVC pipe can also be used (Fig. 5). The spawning structure should serve a dual purpose, providing shelter in addition to adequate space for spawning behavior. Often the spawning structure is placed in the center of the tank to discourage spawning



Figure 5. Clownfish broodstock tank setup used at Roger Williams University (RWU) Center for Economic and Environmental Development (CEED) Marine Laboratory (Bristol, RI). Photo credit: Elizabeth Groover.

on tank walls. To promote year-round spawning, a 14 hour light/10 hour dark photoperiod is recommended.

Broodstock diet is very important for proper development of the gonads and subsequent egg quality. A varied, high quality diet, may consist of a commercially available pellet or custom formulated gel diet offered several times per day until satiation. Further supplementation with fresh or frozen fish eggs, *Mysis* sp. (mysis shrimp), or *Chironomus* sp. (bloodworms) is encouraged and live foods such as enriched adult *Artemia* or *Mysis* sp., may also be included. Furthermore, investment in quality broodstock nutrition will help to accelerate conditioning time and promote high quality spawns. The diet should contain at least 40 percent protein, be fortified with essential fatty acids, vitamins, and minerals. Dietary protein sourced from fish meal is preferable to protein derived from plant matter. Both underfeeding and overfeeding should be avoided to accelerate conditioning while maintaining appropriate water quality.

Establishing a strict maintenance and feeding routine minimizes stress and supports broodstock conditioning. Tank maintenance should be kept to a minimum to reduce disturbance of the broodstock during this time. Uneaten food and waste should be removed daily via siphoning to reduce accumulation of nitrogenous wastes unless broodstock tanks have been engineered to be self-cleaning.

When obtaining clownfish broodstock, it is best to start with sexually immature males. These younger fish will adapt more readily to a new environment with minimal stress as compared to full grown adults. Starting with young male fish will facilitate the natural formation of a social hierarchy and reduce the risk of accidentally pairing two females, which commonly results in aggression and possible injury or death.

There are several methods to successfully pair clownfish. One method is to keep a large group of young juveniles in a tank with a single spawning substrate and allow a pair to form which will begin to guard the substrate. Another method is to place four fish into a tank, two small and two large and allow a single pair to form. Irrespective of pairing method used, it is very important to keep careful watch for aggression and remove the remaining fish once a pair is formed. Only one pair of clownfish should be housed in a single broodstock tank. Once a pair is formed it may take several weeks to months before the first spawn and longer to see consistent high quality spawns. Spawning generally occurs year-round approximately twice a month in captivity, roughly twice the frequency of reproduction in the wild.

The dominant female initiates typical spawning behavior, often cleaning and defending a chosen nest site

several days prior to the event. Spawning usually occurs between noon and dusk. The female will nudge the male, sometimes quite violently, towards the spawning site where they both engage in site cleaning, nipping, chasing, and submissive “twitching” behavior. A female with a partially or fully exposed ovipositor (a tube-like organ used to deposit eggs) indicates that spawning will occur within the next day or so. When the female is ready, she will make ventral passes along the nest site, depositing lines of eggs which adhere to the substrate with fine filaments, ultimately forming a condensed, circular mass of eggs. The male will follow close behind, fertilizing the eggs. The spawning event typically lasts between 1.5 and 2 hours. Parental care is a hallmark of clownfish, exhibited primarily by the male “mouthing” and fanning eggs with pectoral and caudal fins to remove fungus, algae, and bad eggs from the nest. Nest size typically ranges from 300 to 600 eggs and is dependent upon pair age, size, and spawning history, as well as environmental conditions. Few widely scattered eggs are often produced by newly formed pairs or may indicate environmental disruption, and tightly packed eggs normally represent good spawns.

Eggs and larvae

Healthy clownfish eggs are elliptical in shape [approximately 0.08 inches (2 mm) long and 0.04 inches (1 mm) wide] and will appear bright orange initially. As incubation progresses, the orange eggs will transition to various shades of grey. During the last few days of incubation, the egg casing will turn transparent, revealing the developing larvae inside with clearly defined shiny silver eyes. Pale or cloudy eggs indicate poor egg quality or disease. Incubation typically lasts around 7 days but can vary depending on environmental factors (e.g., temperature, and water quality) and broodstock nutrition. Larvae can be allowed to hatch inside the broodstock tank or the spawn can be removed and allowed to hatch in a separate system. There are inherent advantages and disadvantages to each hatching method.

Larvae allowed to hatch inside broodstock tanks risk predation from the parents and could inadvertently end up in filtration equipment. However, allowing eggs to receive the maximum amount of parental care can reduce risk of disease. Direct handling of larvae should be avoided; however, passive collection can be used to transfer the larvae to a new tank. Newly hatched larvae are positively phototactic and can be concentrated using point source lighting and either manually siphoned or directed using plumbing into an adjacent tank. This method usually results in less than 100 percent of larvae successfully transferred.

Eggs that are removed from the broodstock system, prior to hatching, generally tolerate handling and changes in water chemistry very well and eliminate the risk of mortality via parental predation and filtration equipment. It is important to allow the eggs to remain in the broodstock tank as long as possible to allow for maximum parental care. When the larvae are clearly visible within the egg, that is a good indicator that hatching will occur that day and the nest can be removed. It should be noted that active breeding pairs can be aggressive towards an intrusive human hand and may bite. Gloves can be worn for protection if desired. Be sure to immediately replace the spawning substrate in the tank with an identical one to reduce broodstock stress. Before adding the spawning substrate with attached eggs to the larval tank for hatching, the area around the eggs should be cleaned of any algae or organic debris and the entire substrate may be disinfected, if desired, by immersion in a saltwater solution of 250 parts per million (mg/L) formalin (37 percent formaldehyde) for 1 to 2 minutes. Alternatively, 25 parts per million (mg/L) Betadine (10 percent iodine) may be used for approximately 5 minutes. The spawning substrate should be gently rinsed in saltwater and set up in the larval tank with gentle, coarse, aeration flowing just past the eggs to simulate parental fanning behavior. To initiate synchronous hatching, the larval tank should be completely darkened by covering with an opaque material (industrial black trash bags are commonly used) around dusk. If the spawn was pulled at the correct developmental time, hatching should occur 1 to 2 hours after the tank was darkened and all larvae should hatch within a 15 to 20-minute time span.

Larval tanks typically range from 26 to 53 gallons (100 to 200 L) in size and can range in shape, material, and color, although all black round tanks are recommended. Individual controls for aeration and water flow in each larval tank should be available, as well as an internal drain or standpipe covered by a small mesh screen to prevent larvae from being flushed out.

Newly hatched clownfish larvae are relatively large [approximately 0.16 inches (4 mm) total length] and well developed compared to most marine species. Functional jaws and eyes, a rudimentary digestive tract, and fins give these young larvae the ability to swim, hunt, and digest prey immediately after hatch. Clownfish larvae hatch with a small yolk sack reserve that is used up within 3 days. Enriched rotifers (*Brachionus plicatilis* or *B. rotundiformis*) are the standard first feed for clownfish and should be maintained at a density of 3 to 10 per milliliter for the first 7 days post hatch (DPH) (see SRAC Publication

No. 0701, *Culture of Small Zooplankters for the Feeding of Larval Fish*). It is best to deliver newly enriched rotifers in small frequent feedings instead of adding large quantities all at once. This will help to preserve the nutritional quality of the enriched rotifers and prevent larvae from ingesting rotifers which have already metabolized the enrichment and are thus nutritionally inferior. It should be noted that calanoid copepods have been successfully utilized in place of rotifers but this feeding practice is not commonly used in commercial production. Newly hatched *Artemia* should be gradually introduced to the larval tank at low densities around 5 DPH (see SRAC Publication No. 0702, *Artemia Production for Marine Larval Fish Culture*). Once most of the larvae have rounded orange stomachs (indicating that they are full of *Artemia*), rotifers can be phased out of the diet. It is also important to begin introducing small quantities of a commercially available microparticulate larval diet (50 to 100 microns) early in development, also around 4 DPH. Increasingly larger particle sizes can be added to the tank as the larvae grow and are weaned off live feeds around 14 DPH. Settlement (characterized by the larvae orienting themselves with the sides and bottom of the tank) and metamorphosis (characterized by development of full body pigmentation, fin rays, scales, and complex gastrointestinal tract) take place 10 to 20 DPH when the larvae are about 0.35 inches (9 mm) in length.

“Greenwater” culture is a common method for raising many marine fish larvae, including clownfish. The addition of microalgae (live or concentrate) to the larval rearing tank has been shown to result in increased survival and growth. The addition of microalgae to the culture environment has several potential benefits, including but not limited to, continued enrichment of live feeds, improvement of visual contrast for increased prey capture, reduction of light intensity, and stimulation of larval gut microfauna and enzymatic synthesis (see SRAC Publication No. 5004, *Phytoplankton Culture for Aquaculture Feed*).

Growout

Intensive culture is the most common and practical method used when growing out clownfish. Clownfish can be moved to a nursery or growout system about two weeks post-metamorphosis and should be stocked into appropriately sized tanks at appropriate densities that account for water quality, system design, and production goals. Around 20 to 30 DPH, clownfish are considered “true juveniles” when they reach a total length of at least 0.5 inches (13 mm). Water quality, food quality, and food

quantity strongly affect growth. Ideal culture conditions and feeding will generally yield marketable juveniles [1.5 to 2 inches (38 to 50 mm) total length] within 3 to 4 months.

The feeding regime during this phase should consist of at least three feedings per day until satiation to achieve maximum growth. Clownfish do not tolerate repeated handling well, therefore, frequently grading or removing fish for individual sale is not recommended. It is recommended that entire cohorts are allowed to reach market size prior to harvesting.

Disease

Many of the common diseases afflicting marine fishes may also be encountered when culturing clownfish. The most common are *Cryptocaryon irritans*, a ciliated protozoan also known as “white spot” or “saltwater ich” (Yanong 2012), *Amyloodinium ocellatum*, a dinoflagellate also known as “marine velvet” (see SRAC Publication No. 4705, *Amyloodinium ocellatum, an Important Parasite of Cultured Marine Fish*), and *Brooklynella hostilis*, another ciliated protozoan also known as “Clownfish disease”. Other protozoan, metazoan, bacterial, and viral diseases may also occur and adherence to good quarantine and biosecurity practices will help to minimize risk. When in doubt always consult a veterinarian or an aquatic animal health specialist for assistance with diagnosis and treatment options.

Marketing and economics

The current marine ornamental trade in the U.S. includes over 1,800 species of reef fish originating from across the globe. Historically, the quality of data regarding the imports of marine ornamentals has been questionable, with reporting discrepancies commonly found between export and import records. However, there is no denying that the U.S. is the largest import market for marine ornamental fish; estimates by the American Pet Products Association put the number of U.S. households with a saltwater aquarium at 2.5 million, with 18.8 million saltwater fish kept as pets. Over a one-year period, from 2004 to 2005, investigators identified approximately 11 million individual imports of marine ornamental fish to the U.S. Estimates are that the U.S. imported more than 2 million members of the subfamily Amphiprioninae, between 2000 and 2011.

Cultured clownfish are marketed wholesale to pet stores as well as via direct sales to end customers. Typical wholesale prices for cultured *A. ocellaris* clownfish range between

\$2.00 per fish to upwards of \$3.50 per fish (size dependent), with color morphs or unique crosses commonly selling for up to \$9.00; though considerably higher prices based on rarity and aesthetic appeal are possible. It is common to see online marketing for ornamental fish, and a number of retailers use almost exclusively online sales to market and sell clownfish. An important consideration, both for aquarium keepers and culturists, is that each cultured clownfish sold into the aquarium trade may result in fewer clownfish removed from a wild habitat. Marketing materials for cultured clownfish often emphasize the reduced impact on natural ecosystems and wild fish.

Conclusions

In comparison to many marine ornamental fishes, aquaculture of clownfish species is a simple and relatively well defined process that has resulted in a significant volume of captive raised fish available for purchase by consumers nationwide. Selective breeding efforts have been quite successful in recent years, resulting in the production of “designer clownfish” which exhibit a wide range of colors, patterns, and morphologies that vary greatly from their wild counterparts and generally result in increased retail value. Continued research into optimization of clownfish production protocols is still needed to increase on farm productivity and efficiency. Although commercial production of clownfish began almost 50 years ago; today clownfish still represent arguably the most popular and easily recognizable marine ornamental species in the aquaculture industry today.

Suggested readings

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This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2016-38500-25752. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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Department of
Agriculture

National Institute
of Food and
Agriculture

The work reported in this publication was supported in part by the Southern Regional Aquaculture Center through Grant No. 2016-38500-25752 from the United States Department of Agriculture, National Institute of Food and Agriculture.