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Research Article

Clown Fishes Breeding in Captivity Using Low Cost Resources and Water Recycling

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Abstract

Marine aquaculture has grown worldwide in recent decades, but around 90% of ornamental marine fish available in aquarium are results of extractive practices. Ornamental fish farming try to supply the indiscriminate collection of native fish, avoiding their extinction. However, marine fish breeding in captivity still walks on a small scale, being an activity dependent on the acclimatization of the species in tanks, ability of the breeder to produce viable eggs, acquisition of large quantities of larvae, and maintaining the juveniles until they become young adults. The cost of the activity is usually quite high, making it difficult for small producers to invest. The possibility of developing new methodologies could help in reducing the maintenance value of aquaria, ensuring a fish breeding at a lower cost than usual. Thus, using three species of clownfish, we intend to disseminate experiences acquired in the reproduction of these species, at a low cost and with great economic viability. Clown fishes were acclimatized in aquarium devoid of high cost equipment. The water used was conditioned through the brine shrimp breeding, allowing filtration and recycling of the water. Through increased food demand, the courtship was induced and the spawning occurred in all three species. The survival rate of larvae and juveniles was significant, and 85% of the offspring was able to reach adulthood. Despite the form of maintenance described here is not the usual one, the results were positive, allowing the reproduction of fish at a low operational cost.

Keywords: Amphiprion; Reproduction; Spawning; Anemone fish; Marine ornamental aquaculture

Introduction

The aquaculture, defined as farming of aquatic organisms confined in a limited space, is an activity that has been developed around the world for hundreds of years [1]. The earliest writings indicate the birth of aquaculture with monoculture of carp by the Chinese for 2000 years BC [2]. On the other hand, the marine fish farming appears to have arisen in Indonesia in 1400 AC with the farming of the *Chanos chanos* fish. It is also curious to report that Aristotle incorporated the ichthyology to the formal scientific study, doing the first taxonomic classification, in which 117 species of fish of the Mediterranean Sea were described accurately, between 335 BC - 322 BC [3]. However, in economic terms, the oldest log known in this sector is due to Chinese treaty of aquaculture " Fan Li on Pisciculture" (475 BC), which defines captive spawning as a profitable business [4].

Aquaculture activity was highlighted in the 70s in several places around the world, being marked by the extractivism of large quantities of fish from rivers and oceans [5]. A similar situation is still found today. The extractivism of ornamental species, especially the marine ones, presents an even more worrying situation: ornamental marine aquaculture is beginning to develop, with only 8% - 10% of the species of commercial value being cultivated [6]. The other species available in aquariums are derived from extractive practices [7], with a significant impact, especially on the coral reefs, which host 99% of the species of economic interest. Although marine ornamental aquaculture has exhibited great expansion worldwide in recent years [5], this activity is still small-scale, and knowledge is often acquired through trial and error practices, in which information is disseminated informally among the aquarists. The marine ornamental fish farming is not an easy activity. It depends on the acclimatization of the species in captivity, the ability of the breeding stock to produce viable eggs, obtaining large quantities of larvae, and maintaining juveniles until they become young adults [8]. The first larval stages, post-hatching, constitute the most critical period for survival of the individuals [8]. Thus, priority issues in the process of maintaining breeding systems should be addressed in order to provide favorable environmental conditions for the development of the first stages of life of the species [9]. In this respect, ornamental aquaculture tries to supply the indiscriminate collecting of native and exotic fish, which could lead to extinction of these animals, as well as profoundly alter the balance of the aquatic ecosystem to which such species belong [7]. Therefore, understanding the reproductive biology of the species in question, as well as the mechanisms involved in the obtaining larvae, has benefits both for breeding on fish farms and for preserving the species in its natural habitat [6]. The first marine fish successfully reproduced in captivity was the clownfish *Amphiprion* sp, considered the most important marine ornamental fish both in the extractive aspect and in the trade in ornamental fish [7], also leading the first place in the ranking of captive breeding of ornamental marine species. Even so, less than 10% of the animals found in trade are from captivity. This is

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due to the high cost of maintaining marine aquariums and equipment used to obtain good water biology. However, new methodologies can help in the reducing the maintenance value of the aquariums, guaranteeing a fish breeding at a lower cost than usual. In this aspect, using the clownfish as experimental model, the present study intends to disseminate experiences acquired in the reproduction of this marine fish, at a low cost and optimal economic viability, which can be applied to other ornamental marine species, so difficult to reproduce and kept in captivity.

Material and Methods

The animals

Couples already formed of 3 species of clownfish - *Amphiprion ocellaris, Amphiprion frenatus e Amphiprion clarkii* - were transferred to 60 liters aquaria, with temperature at 26 (±2)° C and pH = 8.1 (±0.1). During 15 days preceding the spawning, each couple was fed four times a day with good quality commercial feed and was intercropped at least once a day with live foods (brine shrimp and / or small crustaceans), and fresh food (shrimp) in order to encourage courtship and spawning.

Conditions and installations of the breeding aquarium

The breeding aquarium was equipped only with sand + aragonite substrate, a small earthen vessel to mimic a shelter provided by sea anemones in the natural habitat, and stones and shells. Saltwater was prepared with tap water and commercial salt for marine aquariums, Instant Ocean® Sea Salt. The salinity was maintained at 35 ppt and the density at 1.025, measured through a densimeter, Soma Salinity Saltwater Tool. No chemicals were used to increase or control the pH of the water. No equipment was used as thermostat or chiller to control the water temperature, which remained in ambient conditions. Only one thermometer was kept to check the temperature and variation over the days. Also no expensive equipment was used for the water sterilization system, such as skimmer, external filter, ultraviolet lamp or ozone. In this way, the filtration system only was consisted by a sump to mechanical and biological filtration. The sump was consisted by 5 partitions, each containing:

- 1st partition: large rocks, where the water enters;
- 2nd partition: thick sponge;
- 3rd partition: perlon and activated carbon;
- 4th partition: ceramic;
- 5th partition: return pump aquarium, through which the water exits.

In addition, fortnightly, about 30% of the water of the aquarium was replaced by an equivalent quantity of water from the aquarium itself [10]. This volume of water, rich in organic particles, was used to breed brine shrimp (*Artemia salina*), which filtered the water naturally after a period of one week (Figure 1). Thus, the filtered water was returned to the aquarium of origin. The replacement water of the aquarium and the water for the preparation of new aquariums was always carried out in this way, with no need for water from reverse osmosis. The only additive used in the water was anti-chlorine substances.

Analysis of eggs

After spawning, the eggs of *Amphiprion ocellaris* were observed in a stereomicroscope (Leica) to verify the development of the embryo until the moment of hatching. The eggs were documented using a computerized image analyzer (Leica LAS Interactive Measurements).

Results

After the acclimation period, in which food was intensified with live foods and with more food, the animals entered into courtship, doing nests inside or near the clay vessel. The couples started courtship and nest building between 5 and 10 days after the onset of intensified feeding. In this period, in all cases, the female began to show a fairly oval abdomen and dilated urogenital papillae. Around 3 to 5 days after the nest was built, the spawning occurred. The number of eggs was 300 to 1000 eggs, depending on the size of the animal, the species and the time elapsed between one spawn and another. *Amphiprion ocellaris* began to spawn 1 to 3 times a month (Figure 2). *Amphiprion frenatus* (Figure 3) and *Amphiprion clarkii* (Figure 4) spawned 2 and 5 times over a year, respectively.

After spawning (Figure 2D, 3C and 4), the parents were kept in the aquarium for at least 08 days, until the larvae hatched, since they have parental care. After the hatching of eggs and total consumption of the yolk sac, the parents were removed from the aquarium, which was transformed into aquarium-nursery. The sump pumps were shut down for 10 days after hatching and subsequently the water flow was reduced to avoid sudden movements of the larvae. The water outlet pipe was wrapped in a fine mesh to prevent the larvae from being sucked into the sump.

About 15 days after spawning (Figure 2E), the larvae began to feed. For this, it was necessary a food adequate for its development and size. Thus, the larvae were fed in the first few days with brine shrimp freshly hatched, decapsulated brine shrimp eggs, commercial feed in powdered form for fish, spirulina powder, saline infusory (green water) and frozen pates from fresh foods (liver of steer, shrimp, fish, egg yolk). Gradually, following the metamorphosis of the larvae, around 15 days post-hatching (25 days post-spawning) (Figure 2F), the feeding can be given in larger size, according to juvenile growth (Figure 2G). The juveniles were fed three times daily up to 60 days post-hatch. After this period, they were fed twice a day and transferred to larger aquariums for higher growth, respecting the stocking density of one juvenile for each liter of water. At 3 months after spawning, juveniles already had 2.5 (±0.5) cm (Figure 2H). The mortality occurred especially in the first month after spawning, mainly in the larval period. About 85% of the offspring manage to reach the juvenile period ((Figure 2I), when mortality reaches close to zero. Captive-born animals were able to form new pairs and reproduce under the same conditions in which they were born (Figure 5).

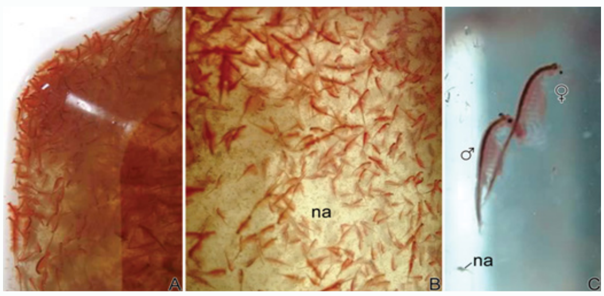

Figure 1: Brine shrimp breeding. A) Adults of brine shrimp (*Artemia salina*) in tank for filtering and recycling water from marine aquariums, rich in algae. B) Detail of A. Note the large number of nauplii (na) of brine shrimp at the bottom of the tank between adults. C) Brine shrimp couple; the adult is 1cm in length.

Embryonic development of *Amphiprion ocellaris*

The eggs of *Amphiprion ocellaris* are large (Figure 6), measuring around 1.5 mm. They are strongly yellowed by the presence of yolk, translucent, demersal and quite adhesive, remaining attached to the substrate by a peduncle on the side of the animal pole (Figure 6). They do not undergo hydration (Figure 6A), but they become quite oval in the first 3 hours after fertilization (Figure 6B). The animal and vegetal pole become visibly separated (Figure 6B). In this period, the zona pellucida stands out from the plasma membrane of the egg, constituting the chorion (Figure 6B). It is possible to visualize a black mass, constituted of melanin in the animal pole (Figure 6B). Around the black mass, oil droplets are observed. Between 6 and 8 hours after fertilization it is possible to observe the formation of an embryonic disc in the animal pole. The cleavage starts. It is meroblastic, resulting in blastomeres (Figure 6C and D) and consequently, in the formation of a blastoderm on the yolk sac. On the 3rd day after fertilization, the formation of the gastrula can be observed (Figure 6E). After the organogenesis period, the optic vesicle becomes evident (Figure 6F and G). The somites differentiate and the embryo becomes larger. Its cranial side migrates to the apical region of the egg (Figure 6H and I). At the end of the first week post-fertilization, the yolk sac becomes smaller and the larvae larger (Figure 6J and L). These show vigorous tail movements (Figure 6M and N) until they can swim, around 10 days post-fertilization.

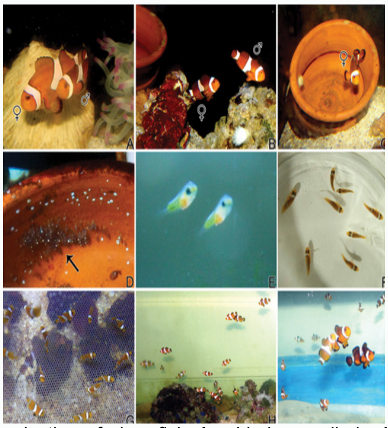

Figure 2: Reproduction of clownfish *Amphiprion ocellaris*. A-B) Clownfish couple. Note the largest female size (9cm) relative to the male (6 cm). B) Clownfish couple. Note the clay vessel on the left side of the aquarium B) Parental care of the female, observing the eggs (arrow). C) Female inside the vessel during the acclimatization to the spawning. D) Eggs development (arrow) after spawning. E) Larvae newly hatched, starting to swim. F) Juveniles with 25 days post-fertilization. G) Juveniles with 40 days post-fertilization. H). Juveniles forming a colony composed exclusively of males with 90 days post-fertilization. I) Clownfish with 300 days post-fertilization.

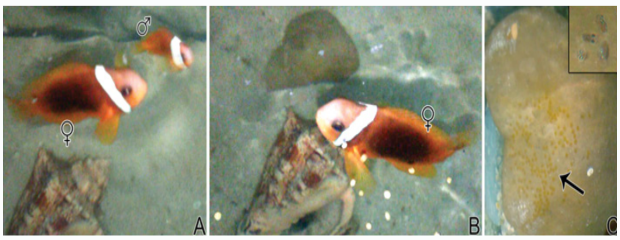

Figure 3: Reproduction of clownfish *Amphiprion frenatus*. A) Clownfish couple in courtship. Note the largest female size (11 cm) relative to the male (6 cm). B) Female swimming on the stone in the background, where spawning occurred. C) Detail of stone, showing the eggs (arrow) post-spawning. Note the embryos adhered to the stone after 7 days post-fertilization (inset).

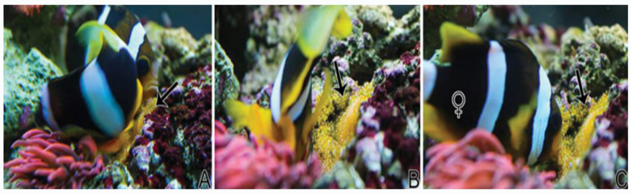

Figure 4: Reproduction of clownfish *Amphiprion clarkii*. A) Clownfish couple at the time of spawning. B-C) Parental care of the female, observing the eggs (arrow).

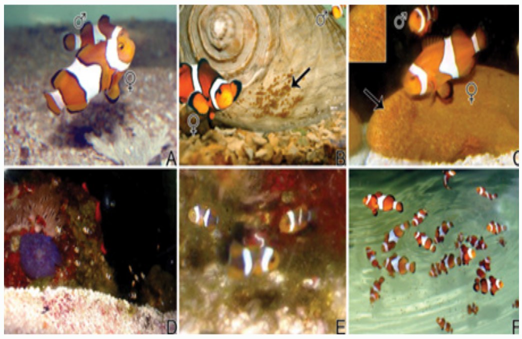

Figure 5: Reproduction of clownfish *Amphiprion ocellaris*, who were born during the experiment and reached adulthood. A) Clownfish couple (2 years old). Note the largest female size (9 cm) relative to the male (6 cm). B) Parental care of the female, observing the eggs (arrow). C) Parental care of the couple, especially the female, with the embryos adhered to the substrate (inset). D-E) Juveniles with 20 days post-fertilization (1 cm). F) Subadults, forming a colony composed exclusively of males with 150 days post-fertilization (4-5 cm).

Figure 6: Eggs and embryo development in different phases of *Amphiprion ocellaris*. A) Egg post- spawning. B) Elongation of the egg, three hours post-spawning . C-D) Stage of two blastomeres (beginning of the cleavage). E) Gastrulation. F-N) Embryonic development. N) Larvae minutes before hatching. Animal pole (av), yolk sac (y), zona pellucida (zp), peduncle (p), blastomere (bl), gastrula (ga), embryo (em). Bar: 200µm (A-C,E,F,H,P-N), 50 µm (D,G).

Discussions

Despite of the form of aquarium maintenance described in this study is not the traditional one used in the breeding of clownfish, it has been very successful in all stages of breeding: acclimatization of the specimens, courtship and spawning, growth of juveniles and reproduction of animals born in captivity. Many aquarists criticize the form used here, arguing that this type of maintenance does not maintain water stability and does not favor aquarium biology [11]. However, since animals can reproduce constantly and grow, reaching their first maturation in the first year of life, we believe that the animals are in good conditions of survival in captivity. In recirculation systems with stabilized parameters, *Amphiprion ocellaris* couples usually reproduce every 15 days and embryo development occurs in about 7 days [12]. In the present study, *Amphiprion ocellaris* couples often also spawned every 15 days, spawning on average at least 3 times a month. Considering a minimum of 300 eggs, a single couple could have 900 larvae a month. If 80% of these larvae survive until 3 months of age, there will be 720 viable juveniles. We consider that these results present favorable economic viability for a small-scale productive system, since in commercial farming for the same species, 80% of couples spawn monthly, with a 20% loss of spawning [13]. However, the cost for the maintenance of these aquaria in the system presented here is much lower than the ones usually used. The viability of the clownfish farming worldwide has a high cost of implementation and significant values for operating costs [14,15], often hindering or discouraging the implementation of a clownfish breeding system in captivity. In this regard, this new way of raising and keeping captive animals can act as an incentive for small producers of the clownfish as well as other ornamental species in captivity. Although the captive breeding does not fully solve the problems of extractivism, it can drastically decrease the collection in the environment. It is known that cultivation alone does not solve the problem, but fish produced in captivity is preferred by aquarists because it presents better quality, easy adaptation in aquarium, and encourages non-capture [9]. In addition to this, the filtration of water by brine shrimp and the recycling of water to the marine aquarium also contributed to an intense reproduction of the brine shrimps and rapid growth of these invertebrates [10]. Therefore, in addition to reusing the water of the aquarium, the brine shrimp used to feed adults, juveniles and larvae, were produced from the system itself, and there were no extra expenses with the acquisition of these invertebrates. In view of the above, we hope that this study can contribute to the success of the breeding of new ornamental mariwne species in captivity, discouraging the collection in the natural habitat through extractivism.

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