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Coral reef fish breeding: the secrets of each species

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Abstract

In recent years, the interest in the trade of tropical fish has increased significantly, with direct negative repercussions on coral reefs and marine ecosystems.

The reproduction and rearing of some of the species most commonly used in the aquarium trade actually represent an economical and ecological tool for broadening development. The present study illustrates the first case ever of a small Indo-Pacific Pomacentridae, *Chrysiptera parasema*, successfully reared in captivity. Eggs were obtained from spawners reared in 80-l tanks under controlled conditions. Spawning began after 3 months: the couples were formed, and eggs were laid after a brief courtship. The male normally guarded the nest and chased away the female if she entered it.

The eggs, about 300 in number, are demersal and elongate ovoidal in shape, measuring approximately 1.2–1.5 mm and coming with a large oil globule. Hatching took place at 28 °C during the first 2 h of darkness, over a total time period of 96 h. A proper diet of enriched PUFA as a first food, combined with a photoperiod of 24L/0D, proved essential for survival of the *C. parasema* larvae.

These results are very promising in terms of both future captive production of ornamental fish and efforts to minimize environmental impact.

Keywords: Reproduction; Nutrition; PUFAs; Embryo development; Photoperiod; *Chrysiptera parasema*

1. Introduction

Tropical and subtropical countries are among the world's largest exporters of ornamental marine species for the private aquarium trade. Many fish collectors in these

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countries employ cyanide to stun tropical fish, making it easier to collect them, but widespread cyanide application harms coral reefs and marine ecosystems and threatens the food sources of the local population. International organizations have been trying for more than a decade to persuade fish collectors that they should use nets instead of cyanide. Unfortunately, the bottom line is that it remains easier and more profitable to catch aquarium fish in an ecologically harmful manner than in an environmentally safe way, and no amount of education or training has been able to change this simple reality. Therefore, in the last few years, a number of different scientists have proposed studying the reproduction of some of the species most commonly used in the aquarium trade for the purpose of rearing them in captivity.

One of the most common of all marine fishes is the yellow-tailed damsel, *Chrysiptera parasema*, which belongs to the large perciform family Pomacentridae. The damsel family contains about 235 species worldwide (Allen, 1975; Nelson, 1984), with most occurring on Indo-Pacific reefs.

The front three quarters of the yellow-tailed damsel's body are bright blue, a color that extends onto the spiny dorsal fin and the spines of the pelvic fins, while the reminder of the body is yellow to orange, fading to a lighter shade at the edges of the unpaired fins.

Like many damselfish, the yellow-tailed damsel deposits demersal adhesive eggs on hard substrates, usually in sheltered areas (Shaw, 1955). The eggs are elliptical and attached by adhesive filaments. Five to six days after fertilization, hatching of the planktonic larvae takes place right after sunset (Foster, 1987; McAlary and McFarland, 1993), when potential diurnal predators have retired to the reef structure. The known range of hatching times for damselfish is 2–7 days. Although the early life histories of many types of damselfish are known, embryological and larval descriptions have been published for relatively few Atlantic species. (Fishelson, 1964; Re, 1980; Re and Gomes, 1982). Today, the list of marine species reared in captivity for purposes other than human consumption includes more than 84 species. The fact that breeding is reported for 26 different species of the Pomacentridae family is worthy of note. This is a significantly higher number of species than for any other family. However, when we look at species that can be reliably reared in large quantities, these include only a dozen species of anemonefish, seven species of Gobiidae, five species of Apogonidae and eight species of Pseudochromidae. Considering that Pomacentridae play an important role in the trade of ornamental fish (Wilkerson, 1998), the aim of this study was to create optimal environmental conditions and proper diet in order to achieve both *C. parasema* reproduction and successful larvae rearing.

2. Materials and methods

2.1. Animals

Eight sexually mature fish, measuring approximately 2.5–3.5 cm, were bought in a pet shop (Acquario di Bergamo, Bergamo, Italy) in January 2000. During the first month, the fish were all kept together in a 120-l tank. Then, when pairs were formed, they were

transferred to 80-l breeding tanks. Males are usually more aggressive than females and present a sharper shape.

The temperature in the breeding tanks was maintained at 27–28 °C, salinity at 28–30 ppt and pH at 8.2. A photoperiod consisting of 13 h of light and 11 h of darkness was provided exclusively by incandescent light from a 25-W bulb suspended 10 cm above the surface of the water in each aquarium. Rocks were placed in the tanks as a surface on which the fish could spawn.

The fish were fed three times a day using a variety of frozen and dry foods.

2.2. Behavioral observations

Notes on reproductive behavior were taken three times a day (8–9 a.m., 12–1 p.m., 5–6 p.m.) during the first 4 months. Attention was focused on the males' choice of territory and on courtship behavior.

2.3. Zooplankton cultures

Different strains of zooplankton were cultured in order to feed the larvae of the *C. parasema* with success during the development stage (Table 1). The first feeding involved rotifers (*Brachionus plicatilis*) at an average dimension of 239 μm. The cultures were maintained using *Chlorella* spp. as phytoplankton at a temperature of 25 °C. The effect of an enriched high polyunsaturated fatty acid PUFA diet was tested. To enrich the rotifers, use was made of commercial SELCON Concentrate (American Marine, USA), an omega-3-fatty-acid-enriching product emulsified with vitamin B12, in accordance with the instructions provided by the company (1.5 ml of SELCON for every 1 l of rotifers culture (300 rotifers/ml) at 25 °C, 8–10 h before use).

The second zooplankton strain, *Artemia* nauplii, was introduced from day 19. Specifically, a small, decapsulated, HUFA-enriched commercial strain was used (INVE Technologies, Belgium) at a concentration of 5 nauplii/ml.

2.4. Collection of larvae

An hour or two before hatching, the rock with the egg clutch was transferred to a 2-l beaker filled with water and placed in a dark room for approximately 75 min where it was gently moved by a very slight flow of water. Following this period, during which hatching took place, the newly hatched larvae were transferred to the larval tank, which presented the same chemical–physical conditions as the breeding tank.

Table 1
Conditions for successful rearing of *C. parasema* larvae

Temperature	28 °C
Salinity	30 ppt
Photoperiod	24L/0D
First food	PUFA-enriched *B. plicatilis* (20 rotifers/ml) from days 1 to 23
Second food	PUFA-enriched *Artemia* nauplii (25 nauplii/larva) from day 19

2.5. Larvae rearing

A 25-l tank was used as the larval tank. The water was neither filtered nor gently aerated, since larvae of this species are extremely sensitive to any turbulence. The sides of the tanks were covered with panels to reduce the reflection of light, while the phytoplankton *Chlorella* spp. and *Isochrysis* spp. were used to "green up" the larval tanks until the bottom of the tank could no longer be seen. Approximately 15% of the water was replaced each morning, and a few drops (2–3) of a 5% KI solution were added twice a day. Egg batches were divided into different groups in order to study the effects of different light/dark regimes and of different diets on the survival rate of the larvae.

2.6. Role of photoperiod and diet

Five egg clutches (about 1400 embryos) obtained from the four couples were pulled and used to estimate the role of photoperiod and diet in the survival of the *C. parasema* larvae.

About 60 ± 5 larvae (group A, in four replications) were maintained at the same photoperiod (13L/11D) as the parents, and feeding schedule will first consist of rotifers, *B. plicatilis*, and then *Artemia*, a super small strain, will gradually be introduced. This should be considered the control group.

The remaining larvae were divided into four groups of 60 ± 5 each (B1, B2 and C1, C2, respectively, each in four replicates) which were reared under two different light regimens: 16L/8D (groups B1 and B2) and 24L/OD (groups C1 and C2).

In addition to light/dark cycles, the effect of a diet rich in polyunsaturated fatty acid was tested by feeding groups B2 and C2 on PUFA's enriched rotifers.

2.6.1. Data analysis

The results were analyzed using ANOVA, followed by Student's *t*-test, with a statistical software package, Stat View 512 + TM (Brain Power, USA). A probability level of 0.05 was utilized to account for the statistical difference between the means. The results are expressed as the means $\pm$ S.D. ($n = 4$) of the data.

3. Results

3.1. Reproductive behavior

The four fish pairs began spawning 3 months after they had been moved into the breeding tank. In all the four couples, courtship began just a week before spawning, with the initiative in courtship being taken by the male, at first by performing a violent swimming motion while clinging with the pelvic cup to the bottom of the aquarium. In the days before spawning, the males actively cleaned the nest site by rubbing it with their pelvic fins and picking off any loose particles or algae with their mouths. The females, during this period, entered the nest several times but did not spawn. On the spawning day, the females entered the nest with a distended venter and participated in a brief

courtship consisting of side to side quivering motions and contact with the males. For their part, the males butted the females in the genital region and slapped them on the head with their caudal fins. After about 30 min, all the females began to spawn, laying the eggs on the clean site; this usually occurred in the early morning, just as the lights were turned on. The eggs, about 300 in number, were immediately fertilized by the males, who usually guarded the nest and chased away the female if she entered it. The embryo development of *C. parasema* occurs within approximately 96 h at 28 °C. During this period, the male stands in front of the entrance to the cave, defending the embryos or fanning them with the pectoral fins and the tail. The parents do not care for the fry after they hatch.

3.2. Embryo development

All the eggs in the nest are encased in a flexible, transparent, conical capsule. At the basal, small end of the capsule is a mass of adhesive threads that anchors the egg to the substrate (Fig. 1A). Around 10 ± 2 embryos obtained from each of the four different egg clutches were used to describe embryo development. At about 23 h, embryonic precursors of the head and the body development are evident (Fig. 1B). At this stage, the embryo inverts itself, so that the head now points towards the distal end of the egg. This new position, with the head of the embryo at the distal end of the chorion, is essential to embryo hatching. At 48 h, numerous myomers form along the mid-body region, and the head, eyes, tail and notochord are evident. The yolk mass is still very large (Fig. 1C). By about 72 h, a vertical fin-fold has developed around the entire posterior portion of the body. Hindgut, eye, brain and nerve cord are in a more advanced stage of development (Fig. 1D). The heart, located in a small sac in front of the yolk sac, in a postero-ventral position to the head, is beating. At 96 h, just before hatching, pectoral fins are well developed and the vertical fin-fold has partially separated into dorsal, caudal and anal fins, although no fin rays are visible (Fig. 1E). The lobes of the brain have developed. The retina of the eye is pigmented, and the embryo is now active, wriggling within the capsule.

3.3. Hatching

Hatching takes place 4 days post-fecundation during the first 2 h of darkness. The tail has wrapped completely around the egg, reaching its distal end. Shortly before hatching, the distal end of the chorion becomes soft and pliable and the movements of the embryo become more frequent and violent until the weakened chorion gives way. The small percentage of the embryos that did not orient properly during the early stages cannot hatch because the proximal end of the egg is not pliable. The larvae that emerge from the capsule still possess a small yolk sac. The mouth is well developed.

3.4. Larval development

At 24 h, the larvae are very active and swim near the surface of the water; the yolk sac is completely absorbed. The first food (*B. plicatilis*) was provided immediately, and we

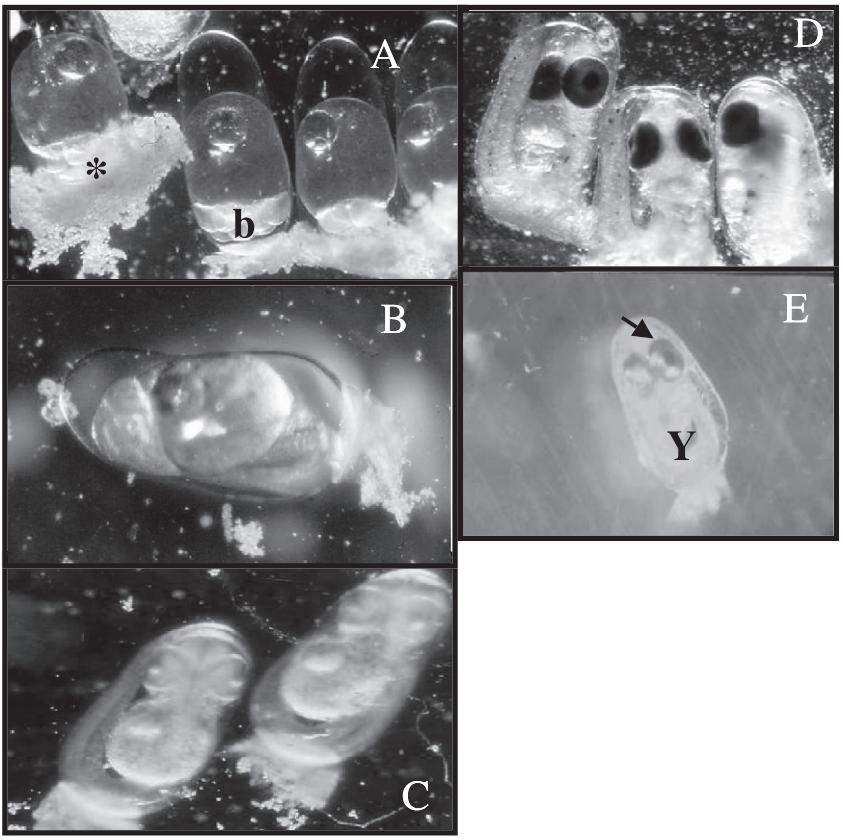

Fig. 1. *C. parasema* embryo development: the mass of adhesive threads (*) that anchor the eggs to the substrate and the first blastomers (b) are visible (A). At 23 h post-fertilization, organogenesis is in an advanced stage, and the embryo inverts itself so that the head is now orientated towards the distal end of the egg. The new position, with the head of the embryo at the distal end of the chorion, is essential to the hatching of the embryo (B). The head, tail and notochord are evident, and numerous myomers form 48 h post-fertilization (C), while the hindgut, eyes, brain and nerve cord are in a more advanced stage of development 72 h post-fertilization (D). Prior to hatching (E), the embryos present a small yolk sac (Y) and a typical metallic pigmented retina (arrow).

estimated that a sufficient density for this larvae was approximately 20 rotifers/ml. More than 28 rotifers/ml leads to the loss of many larvae due to oxygen depletion. In fact, in this species, oxygen levels below 5 ppm represent a critical point for larval survival.

Pigmentation is very light. Body proportions and pigmentation change dramatically during development: on day 15, the body becomes very deep, with a fairly heavy pigmentation, and the first caudal rays are evident from day 12. The pigmentation of the postanal body is characterized by distinct scattered, stellate melanophores along the ventral and lateral midlines. From day 8, a dark ring is visible near the tail.

On day 19, the body is deeper and rounder, especially in the stomach area. Rays are now present on the dorsal, pelvic and caudal fins, and the body is less transparent. On days 21–24, the larvae are on the verge of entering metamorphosis. There is distinct dorsal blue

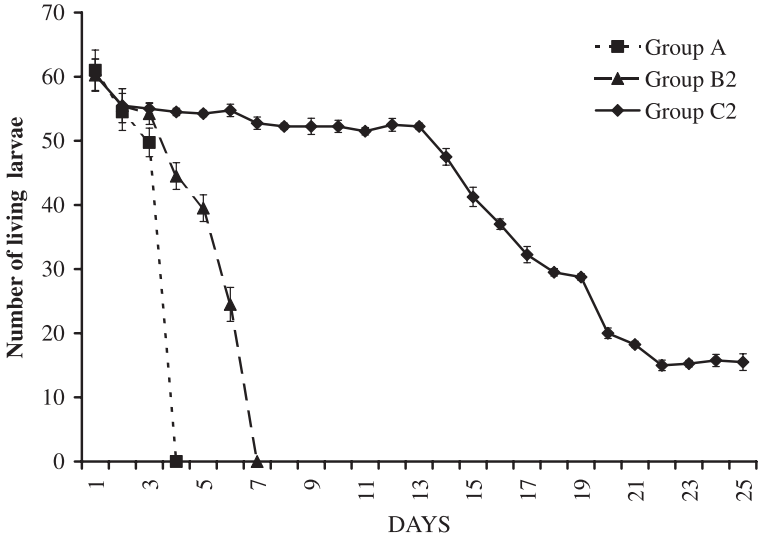

Fig. 2. The effect of different photoperiods on *C. parasema* larval survival when enriched first food was used. Larvae reared using the natural photoperiod (13L/11D) survived until day 3 (group A), while larvae reared using a 16L/8D photoperiod survived until day 7 (group B2). Larvae reached metamorphosis only when an enriched first food was combined with a 24L/0D photoperiod (group C2). A larvae survival rate of 25% was observed.

pigmentation, and on day 25, a yellow tail is visible. During these same days, the larvae move from the surface of the water to the bottom of the aquarium. Due to their sensitivity to light, no photo documentation is available.

3.5. Effect of photoperiod and diet on larvae survival

Our data clearly show that enriched initial food is essential to larval survival. The most interesting data demonstrate that the survival rate changes dramatically when larvae fed on enriched rotifers are reared under different light/dark conditions. As shown in Fig. 2, larvae survived until day 3 when reared under a 13L/11D photoperiod, until day 7 when a 16L/8D regimen was used, and they reached metamorphosis only under a 24L/0D photoperiod, with a juvenile survival rate of 25%.

4. Discussion

Reproductive development can be divided into several distinct sequential processes: gonadal growth, development, final maturation and spawning. If suitable husbandry and appropriate environmental variables are provided, many species undergo gonadal maturation in captivity. In the course of this study, therefore, particular attention was focused on the selection of healthy individuals (Melville and Griffiths, 1997), on the rearing tank size (Ostrowsky, 2000) and on providing the fish couples with a suitable substrate and environment in which to spawn naturally.

Our data show that *C. parasema* needs a 3-month period to start spawning. As in the case of other Pomacentridae, it is the male that guards the egg clutch. More specifically, this is a *k*/*r* strategy species. In fact, the parents take care for the fry until their hatching (*k*), each female deposits hundreds of eggs each 7-day period, the embryo development is very rapid, the larvae are very small and the yolk sac is sufficient to sustain them only during the first 24 h (*r*).

Considering that the major portion of the yolk sac is normally used up during the initial stages of development, it is essential that the larvae immediately be provided with a suitable first food. As described under Section 2, different live foods were provided during development. It was estimated that each larva needs to consume roughly 500–1000 rotifers each day (Wilkerson, 1998). Unfortunately, it is not enough to deposit 1000 rotifers per larva into a rearing tank and simply assume that each larva will find its quota. With this in mind, the rotifer density was kept very high on the first day after the *C. parasema* larvae hatched, ensuring that the larvae would see them frequently. A 20 rotifers/ml density provides the larvae with enough rotifer sightings to realize that rotifers may be edible, and it also provides the larvae with abundant practice targets on which to sharpen their predatory skills. In this study, a great deal of attention was focused on the effects of diet and photoperiod on larvae survival. In particular, we found that no larvae will survive more than 48 h after hatching unless an enriched first food is used. In fact, proper rotifer nutrition and enrichment represents the underlying foundation for the successful rearing of larvae rests. Starving or poorly nourished rotifers are inadequate as first food. In fact, larvae do not survive if fed on rotifers which, in turn, were fed only on algae. The nutritional requirements of marine fish are more rigorous than those of freshwater fish, and so the rotifers must be fed with the nutrients that are needed for the larval development. Enriched rotifers were added to the larval tank twice a day (8 a.m. and 5 p.m.), with this cyclical food density being designed to help them become more efficient food gatherers.

Numerous studies show that a diet rich in PUFAs during the early larval stages is extremely important for optimal nervous system function, in addition to being essential to the development of the brain and to the maintenance of its functional efficiency in adults (Harrocks and Yeo, 1999).

In fact, PUFA-deprived *Solea solea* juveniles show significantly higher mortality when exposed to a combination of low temperature and low salinity, as well as to high temperature and to hypoxia (Logue et al., 2000). What is more, Radunz-Neto et al. (1996) estimated that the best survival and growth for common carp (*Ciprinus carpio*) larvae come from feeding on an enriched $n-3$ fatty acid diet. Similar results were observed in sea bass (*Dicentrarchus labrax*) fed on an essentially fatty acid (EFA) diet (Carnevali et al., 1998). Indigenous tropical brackish water zooplankton, which is normally the first food for most of the reef fish larvae, has a very high content of polyunsaturated fatty acids, which are necessary for the growth of these larvae (Lokman, 1993). Black panels on the aquariums and phytoplankton were used to reduce the "head-butting syndrome" of the fish and to improve water quality, since the algae serve as a nutrient pool (Job et al., 1997). The daily renewal of the water and the addition of KI provide the necessary amount of I_2 for metamorphosis, an event which is strictly correlated to the action of thyroid hormones (Wilkerson, 1998).

C. parasema larvae growth faster and survive with an extended photoperiod of 24 h of light. Under these conditions the fish fed for longer periods of time and yielded higher rates of growth and development. Our data are supported by different works on black porgy (*Mylio macrocephalus*), gilthead sea bream (*Sparus aurata*) and rabbit fish (*Siganus guttattus*) (Kiyonon and Hirano, 1981; Tandler and Helps, 1985; Duray and Kohno, 1988). In contrast, other authors (Fuchs, 1978; Barlow et al., 1995) have reported an optimal growth rate with a 16- or 18-h period of light. This wide range of results suggests that light/dark conditions should be optimized for the specific families and species of fish. The most interesting result of this work is that *C. parasema* can indeed be successfully reared in captivity, as long as the chemical–physical conditions, photoperiod and food chain are optimized, as in this study. In this context, a diet suitably rich in PUFAs and a 24L/0D lighting cycle are indispensable to successful rearing of *C. parasema* in captivity.

5. Conclusions

The results obtained in the present study strongly suggest that a scientific, rigorous approach is needed in order to utilize breeding protocols and successfully rear tropical fish. The information contained here in may represent a starting point for the reproduction of the different species involved in the aquarium trade.

The optimization of breeding and rearing protocols is fundamental to further development of a tropical marine aquaculture.

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