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Aquaculture 250 (2005) 175–182

Aquaculture

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Breeding, rearing and feeding studies in the cleaner goby *Gobiosoma evelynae*

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Received 14 December 2004; received in revised form 24 February 2005; accepted 28 February 2005

Abstract

Breeding and rearing some of the species most commonly used in the aquarium trade actually represent an economical and ecological tool for broadening development, thus the present study investigates captive breeding and rearing of a small goby, the cleaner goby *Gobiosoma evelynae*.

Egg clutches were obtained from two couples maintained in 100-L tanks under controlled conditions. Eggs were laid in PVC pipes and the male normally guarded the nest until the fry hatched.

Hatching took place 168 h post-fertilization at 25 °C. Larvae were divided into different experimental groups and fed on different HUFAs enriched feeding combinations: naked *Euplotes* sp. ciliates and small rotifers *Brachionus rotundiformis* (Group A), small rotifers *B. rotundiformis* and larger ones *Brachionus plicatilis* (Group B) and larger rotifers *B. plicatilis* solely (Group C).

Significantly higher survival rates (50% juveniles) were observed in larvae fed on the naked ciliate *Euplotes* sp. and smaller rotifers *B. rotundiformis* with respect to larvae fed on the larger one *B. plicatilis* (10% juveniles). In larvae fed on the smaller rotifer *B. rotundiformis* followed by the larger *B. plicatilis*, a 35% survival rate was observed. From these feeding studies, it is evident that significant differences in survival rates are already evident from day 3 post-hatch, indicating that marine ciliates are the key organism to improve *G. evelynae* larvae survival and thus an alternative food source to copepod nauplii and rotifers.

Keywords: Coral reef; Fish larvae; Rotifers; Ciliates; Reproduction

1. Introduction

With the increased popularity of aquariums in households in many parts of the world, ornamental fish play, today, a growing role in the international

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doi:10.1016/j.aquaculture.2005.02.057

fish trade. The total value of wholesale ornamental trade is estimated at close to US$ 1 billion, and retail trade about US$ 3 billion.

Many fish collectors in tropical and subtropical 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 source of the local population. Therefore, in the last few years, a number of scientists have studied the reproduction of some of the species most commonly used in the aquarium trade for the purpose of rearing them in captivity (Thresher, 1884; Riley and Holt, 1993; Holt, 2003; Olivotto et al., 2003, 2004, in press).

Gobies are species of keen interest for the aquarium trade, especially for their nice colors, habits and peaceful behaviour in the tanks. Moreover, some of them have cleaner habits, making them useful for ectoparasite control. They are typically elongate, small and often cryptic fishes that abound in mangrove areas, sand and mud flats, tropical freshwater lakes and streams and in many shallow temperate marine areas. The suborder Gobioidei reaches its peak in diversity and abundance, on coral reefs. Easily the largest single group of living fishes (more than 200 genera), gobies alone constitute a major segment of the entire reef-fish fauna (Thresher, 1884). A number of families are variously recognized in the suborder, only two of which, the Eleotridae and Gobiidae, are common on the reef. Currently, many workers simply treat all reef-associated gobies as members of a single family, the Gobiidae. The snout is usually pointed, the mouth is small and most of them are benthic (Bauer and Bauer, 1975).

One of the most common aquarium gobies is the cleaner goby, *Gobiosoma evelynae*, a species that usually lives on coral reefs with clear waters, preferably in oceanic, of the Western Atlantic insular areas (Bahamas and the Lesser Antilles to the Northern coast of South America). This species presents a yellow stripe in front of each eye, joining near the snout tip to form a V. The blackish body is crossed along each side by a yellow stripe. The mouth is inferior and they usually feed on ectoparasites or zooplankton.

Like many gobies, the cleaner goby produces demersal adhesive eggs that are characteristically and nearly universally attached to the roof of a small cavity. Courtship and spawning have been described for different species (Bauer and Bauer, 1974; Valenti, 1972); the eggs are usually elongate, with an average size of 1.1–3.3 × 0.5–1.0 mm. Eggs' color ranges from transparent to yellow, depending on the yolk color. Embryo development lasts from 5 to 6 days depending on the temperature and hatching, as previously observed in other species, takes place right after sunset when potential diurnal predators have retired to the reef structure (Fishelson, 1964; Re, 1980; Olivotto et al., 2003, in press).

In natural habitat, first feeding marine larvae usually feed on a wide variety of micro-zooplankton including protozoans (tintinnids, ciliates), dinoflagellates and especially copepods eggs and nauplii (Holt and Holt, 2000; Riley and Holt, 1993). The densities of copepod nauplii may be too low to totally satisfy larval growth requirements in the wild (Arthur, 1977; Hunter, 1981). Moreover, marine fish larvae, which are usually characterized by a small mouth, may suffer restrictions in available food size (Doi et al., 1997), thus, in the wild, an alternative food source to copepod nauplii may be represented by ciliates. Ciliates are often more abundant in costal waters (Uye et al., 1996) than copepod nauplii and most of them are of a similar or smaller size than copepod naupliar stages.

Rotifers and brine shrimp are the most widely used live food items in marine fish culture, but they are not always the best first food for fish larvae (Holt, 2003).

Since Gobiidae are part of the list of the species that can be reliably reared in captivity in large quantities, and they play an important role in the trade of ornamental fish, the aim of this study was to describe *G. evelynae* breeding conditions and the optimization of the first feeding using different zooplankton strains.

2. Materials and methods

2.1. Animals

Four sexually mature fish, measuring approximately 3–3.5 cm, were bought in a pet shop (Fauna Esotica, Civitanova Marche, Italy) in February 2004.

During the first month, the four fish were kept all together in a 200-L tank. Then, when pairs were formed, they were moved to 100-L breeding tanks. Males are usually more territorial than females.

The temperature in the breeding tank was maintained at 25 °C, salinity 30 ppt, pH 8.2 and NO_2 and $NH_3 < 0.03$ ppm. A photoperiod of 13 h of light and 11 of darkness was provided exclusively by two 30-W incandescent lights suspended 20 cm above the water surface. PVC pipes were placed in the tank as a surface on which the fish could spawn.

The fish were fed twice a day using *Artemia* sp., frozen plankton and chopped fish and shrimps.

2.2. Behavioural observations

Notes on reproductive behaviour were taken three times a day (8–9 a.m., 12–1 p.m., 5–6 p.m.) during the first 3 months. Attention was focused on the males' courtship and parental care behaviour.

2.3. Sampling of embryos and larvae

A subsample of the embryos and the larvae, obtained from the two different couples (three replicates each), was taken at 24, 48, 72, 96, 144, 168 h post-fertilization (p.f.) and 2, 17, 25 and 32 days post-hatch (at 9 a.m.), respectively. The main developmental stages were photographed under the microscope.

2.4. Zooplankton cultures

Different species of zooplankton were cultured in order to feed the cleaner goby larvae during the larval phase.

Two different rotifers species (*Brachionus plicatilis*, *Brachionus rotundiformis*) characterized by an average size of 239 and 160 micron respectively, and *Euplotes* sp. ciliates (average size 100×85 μm) were cultured on *Nannochloropsis oculata* at 30 ppm salinity and 25 °C temperature.

Super small *Artemia* sp. nauplii (INVE Technologies, Belgium) were introduced in the larval tank from day 15.

All the zooplankton species were enriched using Algamac 2000 (Aquafauna Bio-Marine, Inc., USA) following the instructions provided by the company (0.5 g/million rotifers; at 25 °C for 8–12 h).

2.5. Hatching

An hour before hatching, that occurs at about 168 h p.f. at 25 °C, the PVC pipe with the egg clutch was transferred to the 20-L larval rearing tank characterized by the same chemical–physical characteristics of the parent's tank. An air stone was gently put into the PVC pipe in order to create a water flow into the pipe and the egg clutch was left in darkness for about 1½ h. After this period, hatching took place. The average hatch-rate for the different clutches considered for feeding studies was $94 \pm 3\%$.

2.6. Larvae rearing

The water in the 20-L larval tank was gently replaced twice an hour by a dripping system. The sides of the tank were covered with black panels to reduce light reflection, while the phytoplankton *N. oculata* was used (50000 cells/mL) to condition the tank.

Larvae were divided into three different groups fed on different zooplankton combinations in order to study the effect of different preys on larval survival.

All the experimental groups were subjected to an extended photoperiod (24 L/0 D).

2.7. Role of different plankton species on larval survival

Nine egg clutches (about 1800 embryos) obtained from the two couples were used to estimate the role of the different live cultured preys on larval survival.

About 200 ± 5 larvae (Group A, in three replicates) were fed for the first 4 days post-hatch on enriched *Euplotes* sp. ciliates (10 ind./mL) and from day 5 until day 18 on enriched *B. rotundiformis* (10 ind./mL).

A second group, Group B (200 ± 5 larvae, in three replicates), was fed from day 1 to day 4 post-hatch on enriched *B. rotundiformis* (10 ind./mL) and from day 5 to day 18, on enriched *B. plicatilis* (10 ind./mL).

The third group, Group C (200 ± 5 larvae, in three replicates), was fed directly from day 1 post-hatch on enriched *B. plicatilis* (10 ind./mL).

In all of the three groups, *Artemia* sp. nauplii were introduced from day 15 until metamorphosis at a concentration of 5 ind./mL.

The three replicates of each group were obtained randomly from the two different couples in order to avoid possible differences in egg quality.

3. 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 of 0.05 was utilized to account for the statis-

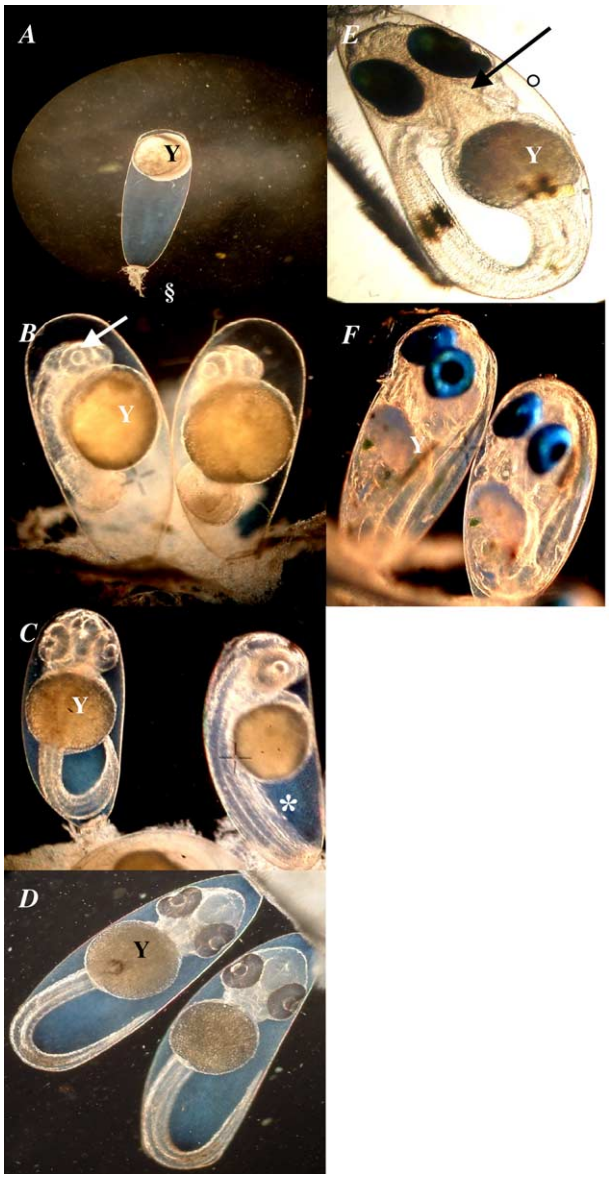

Fig. 1. Cleaner goby embryo development (10× magnification). 24 h p.f. (A), embryonic shield was evident as were a large yolk sac (Y) and adhesive filaments (§). 48 h p.f, (B), lenses appeared (arrow) while the anal pore (*) and a yolk mass reduction were observed 72 h p.f.(C). 96 h p.f. (D), the retina appeared heavily pigmented while pectoral fin primordials (°) and branchial arches (arrow) were evident 144 h p.f. (E). 168 h p.f. (F), embryos are ready to hatch and the typical metallic eyes are evident.

tical difference between the means. The results are expressed as the means ± S.D. of the data.

4. Results

4.1. Reproductive behaviour

Both couples started spawning 3 weeks after they were moved to the breeding tank. Imminent spawning was preceded by an obvious swelling of the female's abdomen and by nest preparation behaviour by the male.

Such preparation, which started 2 days before spawning, usually consisted of cleaning and clearing an area on the roof of the PVC pipe by biting its surface and rubbing the anal fin and venter against it. Spawning always occurred between 10 and 11 a. m. and at this time, the male "enticed" the female to the nest by swimming back and forth between her and the nest site. Successively, the male started approaching the female by vibrating vigorously and after about half an hour, the female followed the male in the nest site and the two fishes started spawning. Spawning occurred with both fishes quivering side by side over the nest site. Females have been observed entering and exiting the nest several times before spawning was completed. Each egg clutch was composed of about 200–250 eggs.

Following spawning, the male tended and guarded the clutch. Female participation was minimal even if at night, she was observed several times sleeping in the nest with the male.

The embryo development of the cleaner goby at 25 °C occurred within approximately 168 h. The parents did not take care of the fry after they hatch.

4.2. Embryo development

All the eggs in the nest were encased in a flexible, transparent, elongated capsule. At the basal end of the capsule was a mass of adhesive threads that anchored the eggs to the substrate. 10 ± 1 embryos for each described stage, in three replicates obtained randomly from the two couples, were used to describe embryo development.

At about 24 h p.f., the embryonic shield was evident and a very large yolk sac was present (Fig. 1A).

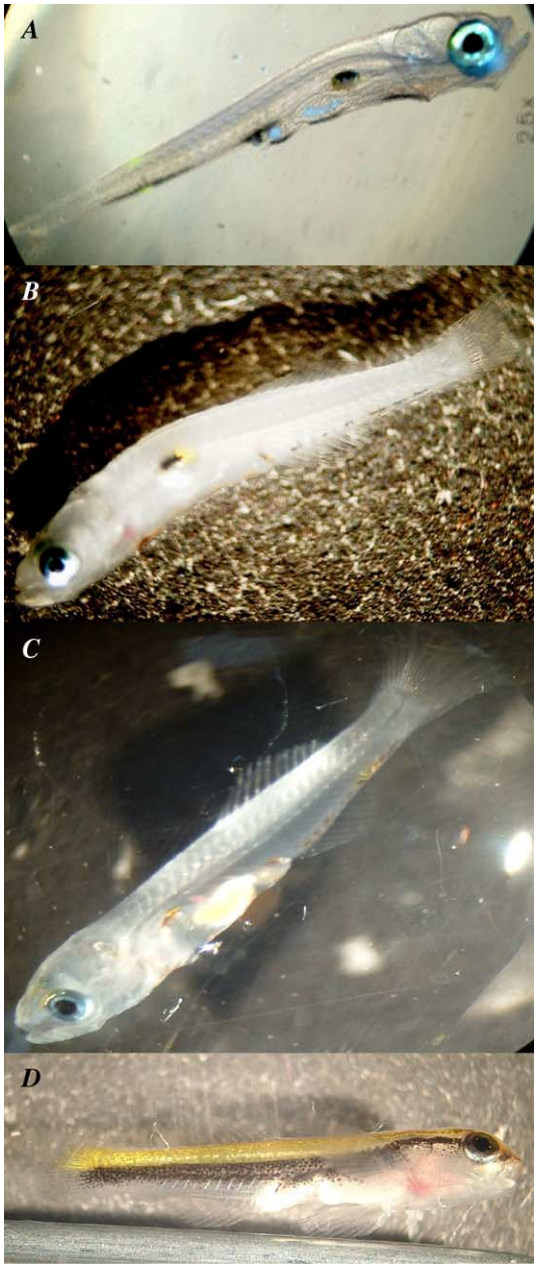

Fig. 2. Larval development of the cleaner goby (5× magnification). 48 h post-hatch (A), larvae were slender and presented a very light pigmentation. Eyes, mouth and gut were well developed. 17 days post-hatch (B), larvae were in a more advanced stage of development and the fin folder had changed in dorsal, pelvic and caudal fins. On day 25 (C), the body was deeper and the larvae were still planktonic. Between days 30 and 40 (D), larvae entered metamorphosis and 2 days after settlement, the typical yellow and black color was evident.

At 48 h p.f., the tail bud projected beyond the large yolk sac. In the anterior part of the embryo, lenses were evident as well as auditory placodes. At this stage, the embryos inverted its position and the head was orientated to the distal end of the adhesive side (Fig 1B).

At 72 h p.f., the heart, located in a small cavity, was beating. The posterior body cavity was prominent and the anal pore was evident. A reduction in yolk mass was observed (Fig. 1C).

At 96 h p.f., aside for an increase in body's length and reduction of yolk mass, some melanophores along the posterior lateral part of the embryo were evident. The retina appeared heavily pigmented by a rusty color (Fig. 1D).

At 144 h p.f., a further increase in body's length was observed especially in the caudal area. Retina was well pigmented and pectoral fin primordials were evident as well as branchial arches (Fig. 1E).

At 168 h p.f., embryos were ready to hatch. The tail had wrapped completely around the egg, reaching its distal end respect to the adhesive side, and the embryos showed the typical metallic eyes, indicating that they were prior to hatch (Fig. 1F). A fin folder was evident and the embryos were particularly active, wriggling within the capsule. This movements and the pliable distal end of the chorion were essential for the larvae to hatch.

4.3. Larval development

10 ± 1 larvae obtained randomly from egg clutches from the two couples, in three replicates for each described developmental stage, were used to describe larval development.

Newly hatched larvae were very active and swam near the water surface; the yolk sac was almost completely reabsorbed. First food was offered immediately the day after hatch.

48 h post-hatch (p.h.), larvae measured approximately 3.7 ± 0.2 mm, were slender and pigmentation was very light. Eyes, mouth and gut and a finfold were well developed (Fig. 2A).

17 days p.h., larvae were in a more advanced stage of development, and the finfold had changed in dorsal, pelvic and caudal fins and fin rays were evident at this time. The body was less transparent (Fig. 2 B).

On day 25, larvae measured approximately 7 mm and the body was deeper and rounder especially in the gut area. The yellow–pink color of the stomach area indicated that larvae were feeding on *Artemia*. At this time, larvae were still planktonic (Fig. 2 C).

Between days 30 and 40 p.h., the larvae entered metamorphosis. During these days, the larvae moved from the water surface to the bottom of the aquarium. The distinct yellow and black pigmentation always appeared 2 days after settlement (Fig. 2 D). Even if it is well known that a specific percentage of the F1 generation of captive bred fish is deformed and/or presents a wrong/incomplete pigmentation, all the juveniles obtained from the different experimental groups presented a perfect look.

4.4. Effect of first feeding on larval survival

A highest juveniles survival rate (50%) was observed in larvae fed on ciliates and *B. rotundiformis* (Group A). Larvae fed on *B. rotundiformis* and on *B. plicatilis* (Group B) showed a 35% survival rate

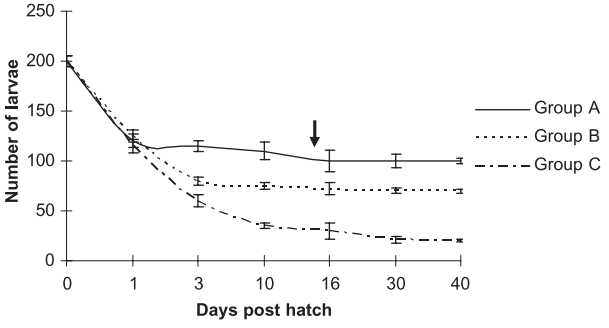

Fig. 3. Cleaner goby larval survival in the different feeding experiments. Survival rate was significantly higher (50%) in Group A fed on ciliates and *B. rotundiformis*. 35% and a 10% survival rates were observed in Groups B and C, respectively, fed on *B. rotundiormis* and *B. plicatilis* and only on *B. plicatilis*. The arrow indicates when *Artemia* sp. nauplii were firstly introduced.

(Fig. 3). In Group C, fed on larger *B. plicatilis* only a 10% survival rate was observed.

In particular, at day 3 p.h., significant differences ($p<0.005$) were observed between Groups A and B and between Groups A and C indicating that ciliates are the key organism to successfully rear this species. At this time, no significant ($p>0.005$) differences were detected within the two groups (B and C) reared using rotifers; differences became significant ($p<0.005$) from day 10.

In all experimental groups, higher mortality was observed on day 1 post-hatch (Fig. 3).

5. Discussion

Providing suitable husbandry and appropriate environmental variables, many species undergo gonadal maturation in captivity. Even if a large number of marine ornamental fish and invertebrates have been spawned, only a few have been successfully reared in captivity (Holt, 2003; Olivotto et al., 2003, in press). In fact, the number of marine ornamental species that can be economically produced on commercial farms today is limited. A critical bottleneck continues to occur at first feeding, when larvae change over from internal yolk stores to exogenous feeds.

First feeding marine larvae feed on a wide variety of marine micro-zooplankton such as protozoans (ciliates, foraminiferans), dinoflagellates, mollusk larvae and copepod eggs and nauplii (Holt and Holt, 2000; Riley and Holt, 1993; Olivotto et al., 2004). Rotifers (*B. plicatilis*) and *Artemia* sp. nauplii are the most widely used live preys in marine fish culture, but they are not always acceptable food for newly hatched larvae. In fact, in the wild, copepod nauplii and copepodites are the natural food of fish larvae (Olivotto et al., in press), but unfortunately, they have not generally been used extensively in aquaculture since they are difficult to culture on a continuous basis.

An alternative may be represented by marine ciliates since they are often present in higher number in the water column than copepod nauplii (Kamiyama, 1994; Uye et al., 1996) and most of them are of a similar or even smaller size than copepod nauplii (Taniguchi, 1977). Naked ciliates such as *Euplotes* sp. offer the advantage to present a small size (100×85 μm) and may be easily cultured applying the same techniques used for Rotifers. Since Rotifers and *Artemia* sp. nauplii are the most widely used live preys in marine fish culture, in the present study, along with these two zooplankton, *Euplotes* sp. ciliates were introduced as live preys and survival rates, in larval cleaner goby fed on three different feeding schedules, were compared.

While spawning in the cleaner goby was fairly straight forward, size of first food offered was found to be crucial in the early rearing of the cleaner goby larvae. Higher survival rate (50%) was observed in larvae initially fed on *Euplotes* sp. ciliates and successively on *B. rotundiformis* rotifers; on the other end, the lowest survival rate (10%) was observed in groups fed exclusively on the larger rotifer *B. plicatilis*. In larvae initially fed using the smaller rotifer *B. rotundiformis* followed by the larger one *B. plicatilis*, a 35% survival rate was observed. This results strongly suggested that the different sizes of the preys offered at first feeding may significantly affect survival rate in this experimental model. In fact, an important attribute in choosing an appropriate larval diet is the body size of the prey in relation to the mouth size of the fish species to be reared (Holt, 2003; Olivotto et al., in press). From these feeding studies, it is evident that significant differences in survival rates are already evident from day 3 post-hatch, indicating that marine ciliates are the key organism to improve *G. evelynae* larvae survival.

In all experimental groups, a significant larval mortality was observed on day 1 post-hatch: this is probably related to physical damage to larvae due to the hatching technique as suggested by Wilkerson (1998).

In particular, this study emphasizes the potentiality of naked ciliates as first food for larval rearing. Similar results were obtained in other studies with both in fresh and marine species (Korniyenco, 1971; Kentouri and Divanach, 1983) where ciliates used as first food significantly enhanced larval survival rates.

The nutritional requirements for long-chain n-3 highly unsaturated fatty acids (HUFAs) for the normal growth and development for marine fish larvae are well established (Sargent et al., 1999; Olivotto et al., 2003). All the zooplankton species used in the present study were enriched in order to optimize their fatty acid profiles.

Moreover, all experimental groups were subjected to an extended photoperiod (24 L/0 D) allowing larvae to feed for longer periods of time and providing in

this way higher growth rates and faster development (Tandler and Helps, 1985; Duray and Kohno, 1988; Olivotto et al., 2003, in press).

6. Conclusions

This study represents an important first step for successful spawning and rearing optimization of the cleaner goby. Spawning was straight forward, but first food size was found to significantly affect survival rate in this species.

Significantly higher survival rates were observed in larvae fed on the naked ciliate *Euplotes* sp. and smaller rotifers *B. rotundiformis* than in larvae fed on the larger *B. plicatilis.* The results obtained in the present study underline the efficiency of naked ciliates as first food for marine fish larvae, thus it may represent a starting point for the use of this species in the aquaculture field.

Acknowledgments

Funding for this study was provided by the "Fondi di Ateneo 2004" to Oliana Carnevali and "Spin off Universitario" Oce. AN. Special thanks to Dr. Roberto Sulpizio, Dr. Francesca Maradonna and Dr. Giorgia Gioacchini for their help with various stages of this project.

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