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Research Article

Use of commercial live feeds enrichment during first feeding period of the barber goby *Elacatinus figaro*

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Abstract. The first feeding period is the most critical phase for the production of marine fish larvae. The utilization of n-3 HUFA enrichment on live feed has improved the results for several species during the larviculture. To evaluate the effect of n-3 HUFA enrichment on survival and growth of the barber goby *Elacatinus figaro* Sazima, Moura & Rosa, 1997, newly hatched larvae were divided in two experimental groups (200 larvae per group, with two replicates each). One group was fed on non-enriched rotifers *Brachionus plicatilis* and the other group was fed with n-3 HUFA enriched rotifers. After 14 days of experiment, survival of larvae fed n-3 HUFA enriched rotifers was three times higher (35.7 ± 3.1%) than those fed non-enriched rotifers (11.1 ± 5.2 %), however this difference was not significant. Growth was faster for larvae fed n-3 HUFA enriched rotifers after the first week of life, but at the end of 14 days, it was no longer significantly different between the two groups (6.09 ± 0.62 and 5.69 ± 0.66 mm). The results of this experiment suggest that barber goby should be fed n-3 HUFA enriched rotifer in order to maximize juvenile production.

Key Words: marine ornamentals, neon goby, larviculture, live feeds enrichment.

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Introduction

Following the world technological and economical development in the last decades, the ornamental fish market is well established and globalized. Home aquariums have made more popular and accessible to common households, increasing the annual consumption of dry goods and live organisms to a multi billion dollars market (Wabnitiz *et al* 2003; Calado 2006).

Similar to the market of marine table fish, where the technology for aquaculture production is available for a small number of species, production of ornamental species is also limited. However, this is a market based on species diversity, commercialized by units instead of weight, and it cannot be supplied with the large production of a few species, as it is a diversified market (Wabnitiz *et al* 2003; Pomeroy *et al* 2006). Therefore, it is necessary to acquire technology for the production of several species of ornamental marine fish.

The development of protocols for cultivation of ornamental fish faces several problems related to the production process, which resemble the difficulties found in the production of juvenile forms of other species of marine fish. The small body size and low fecundity of marine ornamental fish species are two of the main problems fish producers have to cope with (Ostrowski & Laidley 2001; Holt 2003; Moorhead & Zeng 2010).

Most marine fish larvae species are produced using live feed during the first stages of development. Rotifers, *Brachionus* spp. and Artemia nauplii are the most common preys used, but despite the advantages on easy production of large quantities and high densities, it is known that these organisms do not have the fatty acid profile required by most marine fish larvae (Sargent *et al* 1999). In order to improve the output of larviculture, studies using enrichment of live feeds with n-3 HUFA have shown positive results during this period of cultivation (Vagelli 2004; Olivotto *et al* 2006; Drillet *et al* 2011).

Several studies on the requirement of n-3 HUFA have shown that these nutrients are essential for marine fish larvae. Its deficiency can result in a poor development of the visual and central nervous systems (Sargent *et al* 1999). It also negatively affects the growth rate, stress tolerance, pigmentation, and ultimately survival (Copeman *et al* 2002; Olivotto *et al* 2008, 2009).

The Family Gobiidae has the largest number of species among marine fish (Nelson 1994). Gobiidae have a good representation in the international market of marine ornamental fish, accounting for 5 to 7% of total sales (Wabnitiz *et al* 2003; Olivotto *et al* 2005). The barber goby *Elacatinus figaro* is an endemic species from the Brazilian coast with high demand in the international marine ornamental trade market (Gasparini *et al* 2005). However, since 2004 it is listed as an endangered species by the Brazilian Institute of Environment and Natural Resources and can no longer be legally collected from the wild (IBAMA 2004).

Initial information related to the reproduction, embryo development and larviculture of the barber goby has been described

recently (Meireles *et al* 2009; Shei *et al* 2010), and high mortality rates were reported during the first feeding period (Shei *et al* 2010). Expecting to contribute with technology for aquaculture production of juvenile barber gobies this work aims to test the effect of n-3 HUFA enrichment of rotifer on growth and survival rate of barber goby larvae during the first feeding phase.

Materials and Methods

Broodstock

Adult barber gobies were collected (permission SISBIO 22859-1) by scuba diving in the Baía de Todos os Santos, coast of Bahia state – Brazil, and transported to the Laboratory of Marine Fish Culture of the Federal University of Rio Grande (FURG).

Barber gobies pairs were kept in five tanks (30 L) connected to a recirculating aquaculture system (RAS) composed of a 50 µ bag filter (OceanTech, Brazil), three 250 W heaters (Marineland, Italy), a protein skimmer (Plaspiral 1000 HSA, Brazil), a trickling biofilter (7 m^2 total surface), a pressurized mechanical filter (30 µ – Acquafiltros, Brazil) and a 55 W UV sterilizer (> 30,000 µW / cm^2 / s, Sibrape, Brazil). All tanks were supplied with a PVC pipe (32 mm diameter and 80 mm length) with an internal plastic film as suitable substrate for egg laying. Photoperiod was maintained at 12 h light / 12 h dark. Temperature and dissolved oxygen (YSI, 55A, USA), and salinity (Atago salinity refractometer, Japan) were measured daily, while pH (YSI, pH 100, USA), ammonia (UNESCO 1983) and nitrite (Bendschneider & Robinson 1952) methods were measured weekly.

Fishes were fed four times per day, twice (8:00 am and 4:00 pm) with commercial dry feed (59 % crude protein, and 16 % lipids, INVE, NRD, Belgium) and twice (12 am and 7:30 pm) with frozen n-3 HUFA enriched Artemia metanauplii biomass. Under these conditions, fishes spawned every 9-10 days and the embryo development lasted 7 days.

Zooplankton culture

Omega 3 HUFA enriched Artemia metanauplii used to feed broodstock were obtained by hatching Artemia cysts (EG, Inve, USA) in 20 L conical tanks. For the DHA enrichment, the nauplii were conditioned for 24 hours with commercial enrichment emulsion (Easy DHA SELCO, Belgium), following the protocol suggested by the manufacturer. Omega 3 HUFA enriched Artemia metanauplii biomass was frozen and offered to broodstock. This process was repeated every two weeks.

Rotifers (*Brachionus plicatilis*) offered to the larvae were cultured on 150 L tanks on Nannochloropsis oculata and commercial dry feed (S.parkle – INVE Belgium), at salinity 30 and 25°C temperature. Omega 3 HUFA enriched rotifers were obtained with commercial formulations (Algamac 2000 – Aquafauna Bio-Marine, Inc, USA) following the protocol provided by the manufacturer.

Larval rearing

Two hours before larvae began to hatch the plastic film with adhered eggs was removed from the PVC pipe and transferred to a beaker (1 L) with gentle aeration until all larvae hatched. Larvae were than counted, measured under a stereomicroscope

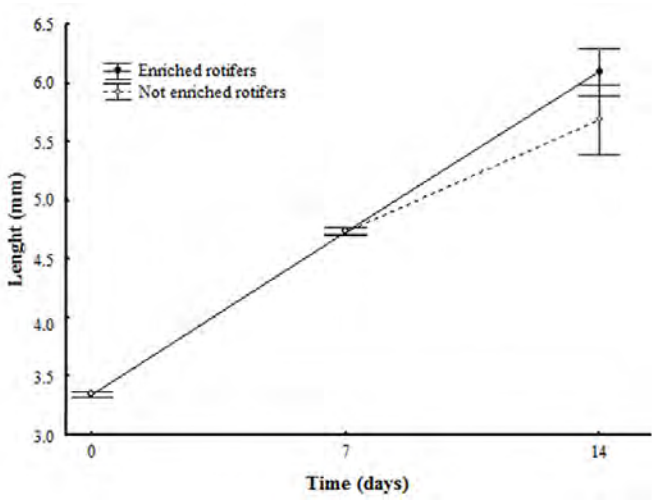

Figure 1. Growth of barber goby larvae *Elacatinus figaro* fed n-3 HUFA enriched and non-enriched rotifers**.**

(WILD, M5A, Germany) and split into four tanks. Each tank was stocked with 200 larvae. Group A fed with non-enriched rotifers and group B fed with enriched rotifers. In both groups rotifers were kept at density of 10 ind / mL, they were replaced 4 times / day in order to assure the quality of the prey. Tanks were maintained with green water (*Nannochloropsis oculata*, 500,000 cells/mL) during the experimental period. Both experimental groups were kept under extended photoperiod 24 L: 0 D.

Larvae were reared in four 90 L cylindrical tanks (black wall and white bottom) connected to a RAS composed by bag filter (50 µ) to retain rotifers from the tanks, two 250 W heaters, a protein skimmer (Plaspiral 800R, Brazil), a fluidized biofilter (bioballs, 5 m^2 total surface) and a 30 W UV sterilizer (> 30,000 µW / cm^2 / s, Plaspiral, Brazil). Larvae from all tanks were measured at 0, 7 and 14 days after hatching (dah). Survival was estimated by counting all remaining larvae at the end of the experiment 14 days after hatch.

Statistical analysis

The results were analysed using Student T test, with Statistic 7.0 Software®. A probability of 0.05 was considered to determinate statistical difference between the means. Results are presented as the means ± standard deviation of the data.

Results and Discussion

Water quality – larval rearing and broodstock

During the experimental period, temperature ranged from 25 to 27 °C, salinity was 32‰, dissolved oxygen > 6 mg/L, pH at 8 – 8.4 and NH_3 and NO_2 < 0.02 mg/L. These parameters are similar to other studies on the culture of barber goby larvae and other *Elacatinus* spp. (Olivotto *et al* 2005; Meireles *et al* 2009; Shei *et al* 2010).

Survival

Three and 4 days after hatching, it was observed a high mortality in one tank of the non-enriched rotifer treatment. Although not significantly different (P=0.055) survival of larvae reared on n-3 HUFA enriched rotifers was more than three times higher (35.7 ± 3.1%) than larvae reared on non-enriched rotifers

(11.1 ± 5.2 %). The high coefficient of variation, 12.1 and 65.9 could have masked the statistical difference on survival rate.

Growth

The standard length (SL) of newly hatched larvae was 3.34 ± 0.07 mm. At 7 dah, larvae fed n-3 HUFA enriched rotifers (4.73 ± 0.09 mm) had grown significantly larger ($P<0.01$) than those reared with non-enriched rotifer (4.5 ± 0.24 mm) (Figure 1). However, at the end of the experiment (14 dah) there was no longer a significant difference of growth for larvae reared on both prey types ($P>0.05$), 6.09 ± 0.62 and 5.69 ± 0.66 mm for larvae fed with n-3 HUFA enriched and non-enriched rotifers respectively.

The development of techniques that improve productivity on marine ornamental species will help to produce organisms that commercially compete with less expensive specimens from the wild and thus reduce the pressure on natural stocks (Wabnitiz *et al* 2003; Olivotto *et al* 2006).

The low fecundity of ornamental species, including barber goby (Olivotto *et al* 2005; Fernando *et al* 2006; Meireles *et al* 2009), make it difficult to run experiments with the adequate number of replicates. The lack of significant difference of survival and growth at the end of the experiment can be the result of the high coefficient of variation observed for both. Nonetheless, the results strongly suggest that barber goby should be fed n-3 HUFA enriched rotifer in order to maximize juvenile production.

Although studies have been conducted to determine nutritional requirements and feeding practices to optimize larval production of a number of marine fish species (Sargent *et al* 1999), studies on marine ornamental species with this type of development are still scarce (Ostrowski & Laidley 2001; Moorhead & Zeng 2010).

The essential fatty acids n-3 HUFA such as DHA and EPA are major components in cell membranes of marine fish and they are also important as source of energy during larval development (Sargent *et al* 1999; Toucher 2003). Studies with several species demonstrate better performance in the larviculture with the use of n-3 HUFA enriched live prey. In fact, growth and survival of seahorses, the false percula *Amphiprion ocellaris* and the Banggai cardinalfish *Pterapogon kauderni* were improved during the early stages of development when fed on n-3 HUFA enriched rotifer and Artemia (Chang & Southgat 2001; Vagelli 2004). Succesfull production of juvenile sunrise dottyback *Pseudochromis flavivertex* was only achieved with the use of n-3 HUFA enriched live food (Olivotto *et al* 2006). The results of this experiment clearly suggest that barber goby larvae should be fed with n-3 HUFA enriched rotifers in order to maximize growth and survival to the juvenile stage.

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