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Short Communication

Anticipation of *Artemia* sp. supply in the larviculture of the barber goby *Elacatinus figaro* (Gobiidae: Teleostei) influenced growth, metamorphosis and alkaline protease activity

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ABSTRACT. The barber goby *Elacatinus figaro* is considered endangered due to overexploitation by the ornamental industry. Farming marine ornamental fishes, especially the threatened ones, can be one of the measures to minimize the pressure on the natural stocks. Among the priority issues for their production is the determination of the most appropriate feeding management. The feeding protocol commonly used in the larviculture of barber goby, when the start of *Artemia* sp. offer occurred at the 18th DAH (days after hatching) (treatment T18), was modified, by anticipating brine shrimp supply in 6 days (treatment T12). Alkaline proteases activity, growth and metamorphosis of larvae were evaluated in both protocols. Juveniles at T12 showed higher weight (0.04 ± 0.001 g) and lower activity of total alkaline proteases (1.3 ± 0.2 mU mg⁻¹ protein) compared to T18 (0.02 ± 0.001 g; 2.8 ± 0.4 mU mg⁻¹ protein, respectively). With anticipation of brine shrimp, the commencing and end of larval transformation was observed earlier (at 24 and 34 DAH, respectively) in comparison to those with the supply of *Artemia* sp. at 18 DAH (27 and 41 DAH, respectively). Thus, the *Artemia* sp. anticipation was beneficial during the larviculture of the barber goby, considering that larvae reached metamorphosis earlier.

Keywords: *Elacatinus figaro*, fish, ornamental, endangered, live food, feeding, aquaculture.

La anticipación del suministro de *Artemia* sp. en la larvicultura del neón gobi *Elacatinus figaro* (Gobiidae: Teleostei) influyó el crecimiento, metamorfosis y actividad de proteasas alcalinas

RESUMEN. El neón gobi, *Elacatinus figaro*, se considera en peligro de extinción debido a la sobreexplotación por la industria ornamental. El cultivo de peces ornamentales marinos, especialmente de las especies amenazadas, puede ser una de las medidas para minimizar la presión sobre las poblaciones naturales. Entre los temas prioritarios para su producción es la determinación de la estrategia de alimentación más adecuada. El protocolo de alimentación de uso común en la larvicultura del neón gobi, cuando se inicia el suministro de *Artemia* sp., que ocurre en el 18° DDH (días después de la eclosión) (tratamiento T18), fue modificado mediante la anticipación de suministro de este microcrustáceo branquiópodo en 6 días (tratamiento T12). La actividad de las proteasas alcalinas, crecimiento y metamorfosis de las larvas se evaluaron en ambos protocolos. Los juveniles en T12 mostraron mayor peso ($0,04 \pm 0,001$ g) y la menor actividad del total de proteasas alcalinas ($1,3 \pm 0,2$ mU mg⁻¹ de proteína) en comparación con T18 ($0,02 \pm 0,001$ g; $2,8 \pm 0,4$ mU mg⁻¹ de proteína, respectivamente). Con la anticipación del suministro de *Artemia* sp. se observó que el principio y final de la transformación de las larvas fue más temprano (a los 24 y 34 DDH, respectivamente), en comparación con aquellos con el suministro de *Artemia* sp. en 18 DDH (27 y 41 DDH, respectivamente). Por lo tanto, la anticipación del

suministro de *Artemia* sp. fue beneficiosa durante el cultivo larval del neón gobi, considerando que la metamorfosis de las larvas se alcanzó antes.

Palabras clave: *Elacatinus figaro*, pez, ornamentales, amenazada, alimento vivo, alimentación, acuicultura.

The barber goby, *Elacatinus figaro*, an endemic marine ornamental fish from Brazil (Carvalho-Filho, 1999) is of interest to the aquarium trade because of its small size, coloration, active behavior and rusticity (Sazima *et al.*, 2000). It is a cleaner fish (Sazima *et al.*, 1996), removing ectoparasites, dead tissue, mucus and scales from the body of other fish and invertebrates (Johnson, 1982; Losey, 1987; DeLoach, 1999). This cleaning behavior is considered of fundamental importance for the maintenance of the equilibrium and health of fish in reef ecosystems (DeLoach, 1999) and in reef aquariums.

Due to an intensive harvest during the past years to the aquarium trade (Gasparini *et al.*, 2005), *E. figaro* has been included in the list of endangered species, and its capture and trade is prohibited by the Brazilian Ministry of Environment (Normative Instruction Number 5 of 21 May 2004), Ministério do Meio Ambiente, (Brasil, 2004).

Farming marine ornamental fishes, especially the threatened ones, can be one of the measures to minimize the pressure on the natural stocks. Among the priority issues for the production of these fishes are the knowledge of the nutritional requirements and the determination of the most appropriate feeding management (Pezzato, 1997; Avella *et al.*, 2007). Since food is the source of energy and nutrients for larval growth and development, the feeding protocol has a strong influence on the development, digestive and assimilation potential of nutrients in fish larvae (Guerreiro *et al.*, 2010). According to preliminary studies with the barber goby, the early supply of either an inadequate or an appropriate live food can alter digestion and food utilization, affecting larval survival and growth (Côrtes, 2009).

In aquaculture, the identification, quantification, and evaluation of the changes in the profile of the activity of digestive enzymes are needed to establish the most appropriate moment to conduct the dietary transition and to assist in choosing appropriate ingredients and developing suitable food strategies in the larviculture and grow out, based on the digestive capacity of fish (Kuz'mina, 1996; Galvão *et al.*, 1997; Fernández *et al.*, 2001; Cara *et al.*, 2002).

In the present study, the feeding protocol commonly used in the larviculture of this species was modified by anticipating the *Artemia* sp. supply in order to evaluate if this anticipation would affect growth as wet weight,

onset and end of metamorphosis (transformation from larvae to juvenile) and activity of total alkaline proteases of juveniles.

The experiment was conducted at the Fish and Marine Ornamentals Laboratory (LAPOM), Federal University of Santa Catarina, Brazil. Two couples of wild *Elacatinus figaro* breeders, captured from the state of Espírito Santo/Brazil with permission for activities with scientific purposes from SISBIO/ICMBio (Number: 22051-2) were used to obtain natural spawning.

Breeders were maintained as described by Meirelles *et al.* (2009). The photoperiod used was 14 h light and 10 h dark. Breeders were fed to apparent satiation twice a day (morning and afternoon) with a varied diet consisting of commercial diets for marine ornamental fish MarineTetra and TetraVeggie (TETRA, Melle, Osnabrück, Germany), marine fish weaning diet NRD (INVE Technologies, Belgium), *Artemia* sp., enriched with commercial emulsion of fatty acids (DHA Selco-INVE Technologies, Dendermonde, Belgium), as well as shellfish, squid, chopped fresh fish and shrimp. Tanks were daily cleaned to remove uneaten food and feces.

Every day, the PVC pipe used as substrate for spawning of each couple was observed, and so the day before hatching was calculated in order to transfer the eggs to 40 L hatching/larviculture tanks with the same physical and chemical water conditions of the parental tanks.

To verify if the anticipation of the brine shrimp, *Artemia* sp., supply in the barber goby larviculture would affect growth and day of metamorphosis of larvae, two different feeding protocols (treatments) (Fig. 1) were performed in triplicate: T18-Standard feeding protocol, when the start of the brine shrimp offer occurs at the 18th DAH (days after hatching) (Côrtes, 2009; Meirelles *et al.*, 2009); T12-Anticipation of the brine shrimp supply in 6 days (12th DAH) from the standard feeding protocol.

After hatching, larvae were kept at the hatching tank at a density of 5 larvae L⁻¹, with microalgae *Nannochloropsis oculata* (0.5-1.0 x10⁶ cells mL⁻¹ of water) and rotifers *Brachionus* sp., until the start of *Artemia* sp. supply, either at 12 DAH or 18 DAH.

After hatching, larvae were reared in aquariums at a density of 5 larvae L⁻¹, with microalgae *Nannochloropsis oculata* at a concentration of 0.5-1.0x10⁶ cells mL⁻¹ of water, for maintenance of rotifers.

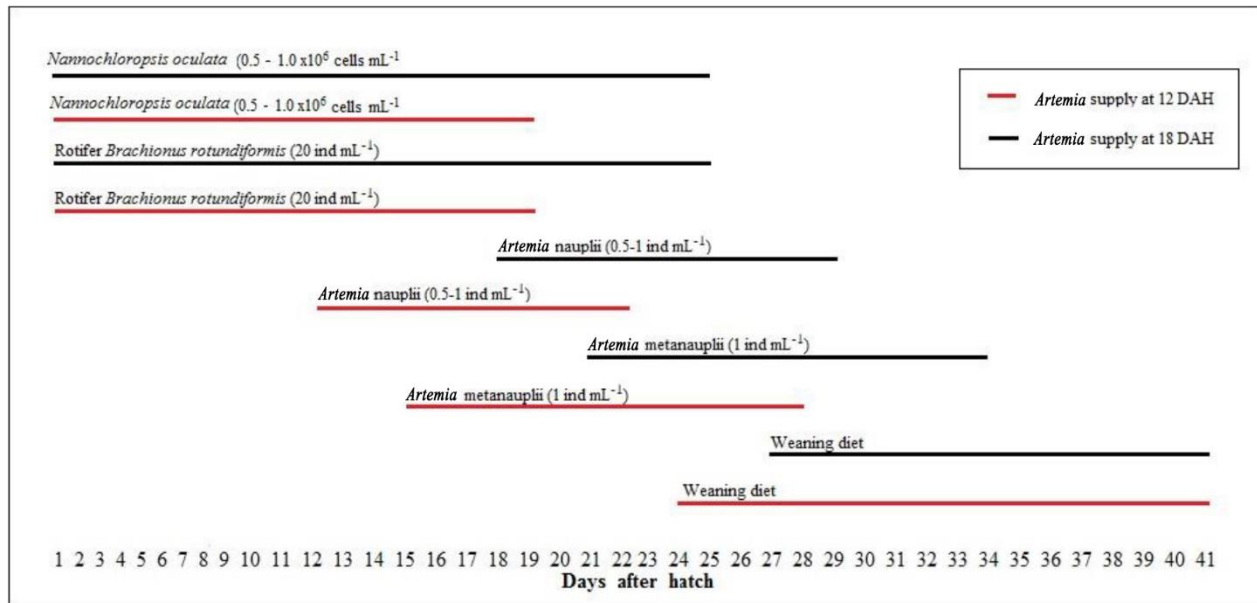


Figure 1. Dietary protocol of barber goby with *Artemia* sp. supply at 12 (T12) and 18 DAH (T18).

Rotifers *Brachionus* sp. (lorica length ranging from 100 to 180 μm) and *Artemia* sp. nauplii and metanauplii were used as live food. Rotifers were cultured at a salinity of 25 g L^{-1} , 26°C, with microalgae *Nannochloropsis oculata* (10^4 - 10^5 cells per individual). *Artemia* sp. nauplii cysts (INVE Technologies, Dendermonde, Belgium) were incubated at 29°C and salinity of 35 g L^{-1} . *Artemia* sp. metanauplii were kept under the same conditions, and after hatching, were enriched with docosahexaenoic acid (DHA) Selco (INVE Technologies, Dendermonde, Belgium), 24 h prior to delivery to the larvae. Dry diet (crude protein 52%; lipid 12%, ash 15%; otohime TDO-A 250 μm ; Reed Mariculture, California, USA) was used for juveniles.

During the experiment, the water temperature was maintained at $26 \pm 2^\circ\text{C}$, salinity $28 \pm 3 \text{ g L}^{-1}$, and water pH at 7.9 ± 0.2 . In each experimental unit, dates of commencement and completion of metamorphosis of the larvae (larvae settled and pigmented) were examined.

Ten fish of 41 DAH were collected per experimental unit (30 fish per treatment) for biometry and quantification of total alkaline proteases activity. This age was fixed for sampling animals as larvae had already metamorphosed into juveniles (settled with typical yellow and black color of the species). In each sampling, fish were caught and immediately immersed in iced cold water, where they were killed by thermal shock. After being dried with paper towel, the biometry was performed. Finally, they were properly packed in aluminum foil and stored at -18°C to determine the

activity of total alkaline proteases. In order to obtain the homogenates for the enzymatic determination, the tail and the head were despoised and a “pool” of 10 juveniles of each experimental unit was used for obtaining a single homogenate.

For the preparation of the enzymatic extract, each replica of samples was individually homogenized in iced-cold distilled water in a ratio of 1:5.5 (tissue: distilled water, w:v), through a van Potter homogenizer for 2.5 min (5 agitations of 30 s with intervals of about 5 min to cooling). They were then centrifuged at $15,550 \times g$ for 15 min at 4°C . The supernatants were used for quantifying the activity of alkaline proteases.

The quantification of soluble proteins of the enzymatic extracts was performed by the method of Bradford (1976) using bovine serum albumin (BSA) (Sigma-Aldrich, USA) as standard. The total alkaline proteases activity of the extracts was measured using the azocasein (Sigma Chemical Co, St Louis, Missouri, USA) hydrolysis, method described by García-Carreño *et al.* (1979). The enzymatic activity was expressed as specific activity ($\text{U mg}^{-1} \text{ protein}$), *i.e.*, units ($[\Delta\text{Abs}_{366\text{nm}} (\text{Test-Control}) \text{ min}^{-1} \text{ mL}^{-1}]$) per milli-gram of protein.

Data were subjected to analysis of variance (ANOVA) with a significance level of 5% (software Statistica 7.0). When present, the statistical differences were measured using the Tukey test. The results were presented as mean \pm standard deviation (SD).

Survival rates did not differ among treatments (mean of 13.5%). Growth of juvenile (41 DAH) barber goby differed between the two treatments ($P < 0.05$), as

larvae with anticipated supply of *Artemia* sp. (T12) showed higher weight (0.04 ± 0.001 g) compared to those that started consuming this live food at the 18th DAH (T18) (0.02 ± 0.001 g). This higher growth at T12 also affected the start of metamorphosis (Fig. 2). Juveniles of 41 DAH fed *Artemia* sp. at the 12th DAH, showed a lower activity of total alkaline proteases (1.3 ± 0.2 mU mg⁻¹ protein) compared to the activity found in fish when *Artemia* sp. was offered at T18 (2.8 ± 0.4 mU mg⁻¹ protein) ($P < 0.05$).

Assuming that larval biomass with anticipated supply of *Artemia* sp. doubled, compared with the traditional protocol, the decline in specific activity of alkaline proteases is not related to a decrease in enzyme synthesis, but might be a result of an increase in tissue protein due to larval growth as cited by Zabonino-Infante & Cahu (2001) and Jimenez-Martinez *et al.* (2012).

The day when *Artemia* sp. was offered to barber goby larvae, significantly affected metamorphosis. In larvae with anticipation of *Artemia* sp. (T12), the commencing and end of transformation was observed earlier (at 24 and 34 DAH, respectively) in comparison to those at T18 (27 and 41 DAH, respectively) (Fig. 2).

Thus, the *Artemia* sp. anticipation proposed in this study was beneficial, once barber goby larvae reached metamorphosis earlier than the commonly used feeding protocol for *E. figaro*, and even in comparison with similar species. In the cultivation of *Elacatinus oceanopsis*, when brine shrimp was added in the larviculture at 15 DAH, fish entered metamorphosis between days 30 and 40 post-hatching (Olivotto *et al.*, 2005). Considering that inert diet is supplied when larvae reached metamorphosis, early metamorphosis of fish would diminish the need of live food.

The success in the hatchery production of marine fish is largely dependent on the availability of suitable live food for feeding fish larvae. Live food organisms contain all the nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acid and hence are commonly known as “living capsules of nutrition” (Das *et al.*, 2012).

The live food contribution to the nutrition, digestion and assimilation process of the barber goby might be considered when defining a feeding protocol for the species. For example, the enrichment techniques commonly used in *Artemia* sp. nauplii increased the availability of highly unsaturated fatty acids to marine fish larvae, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), essential components in

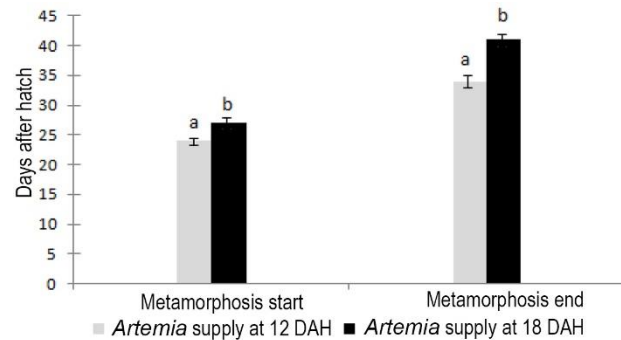


Figure 2. Start and end day of the metamorphosis (mean \pm SD, $n = 10$) of the barber goby in larviculture with beginning of *Artemia* sp. supply at 12 (T12) and 18 DAH (T18). Different letters in the same parameter evaluated indicate significant differences ($P < 0.05$).

the diet (Han *et al.*, 2000). Barroso (2010) demonstrated that *Artemia* sp. has greater amounts of incorporated fatty acids when compared to rotifers, in the larviculture of the fat snook *Centropomus parallelus*, using the same feeding protocol of this study. Possibly, the greater availability of fatty acids in brine shrimp caused an increment in growth in barber goby larvae in which *Artemia* sp. supply was anticipated. The supply of live food with administration of these fatty acids has also been shown to increase the growth and development in ornamental species such as in yellowtail damselfish *Chrysiptera parasema* (Olivotto *et al.*, 2003), in the goby *Elacatinus evelynae* (Olivotto *et al.*, 2005) and in clownfish *Amphiprion ocellaris* (Avella *et al.*, 2007).

Furthermore, because of its greater size when compared with rotifers, *Artemia* sp. motility by the digestive tract may cause mechanical stimuli by increasing the peristaltic movements, which triggers the larval digestive processes (Tandler & Kolkovski, 1991).

In the protocols for marine fish larviculture, the beginning of the use of *Artemia* sp. nauplii (450-700 μ m) is indicated when the larvae are able to consume food larger than rotifers (80-340 μ m) (Côrtes & Tsuzuki, 2012). It is important that the introduction of larger food during fish developing is supplied at the right time, because there is a moment when the use of rotifers is not most beneficial for the larvae, *i.e.*, when the energy spent by larvae to capture food is not compensated by the energy contained in it.

Consequently, in the larviculture of barber goby, the anticipation in the supply of brine shrimp caused higher larval growth and an advance in their development and, as a consequence, their earlier metamorphosis to juvenile.

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