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Research Article

Inclusion of copepod *Acartia tonsa* nauplii in the feeding of *Centropomus undecimalis* larvae increases stress resistance

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ABSTRACT. This research represents the first result of studies of the common snook *Centropomus undecimalis* larvae from broodstock matured in captivity in Brazil. The aim of this study was to evaluate if the inclusion of *Acartia tonsa* nauplii improves stress resistance of common snook larvae. The larvae were fed with: rotifers *Brachionus plicatilis* (10 to 15 mL^{-1}); *A. tonsa* nauplii (0.25 to 0.5 mL^{-1}) and rotifers (5 to 7.5 mL^{-1}), and *A. tonsa* nauplii (0.12 to 0.25 mL^{-1}). The average percentage of survival of the treatments was 11.9%. At 20 days of age, larvae were subjected to thermal stress. Subsequently, the stress resistance was evaluated. Common snook larvae fed *B. plicatilis*+*A. tonsa* reached a higher weight and length (7.5 ± 0.00 mg and 9.1 ± 0.23 mm, respectively) and resisted more heat stress (87.4%) than larvae fed other foods, indicating that the feed mixture is satisfactory as a starter diet for larvae of common snook. However, more research is needed to confirm these results.

Keywords: crustacean, common snook, larviculture, live feed, marine fish, aquaculture.

La inclusión de nauplios del copépodo *Acartia tonsa* en la alimentación de larvas de *Centropomus undecimalis* aumenta su resistencia al estrés

RESUMEN. Esta investigación constituye el primer resultado de los estudios de larvas de róbalo blanco *Centropomus undecimalis* a partir de reproductores maduros mantenidos en cautiverio en Brasil. El objetivo de este estudio fue evaluar si la inclusión de nauplios de *Acartia tonsa* mejora la resistencia al estrés de las larvas de róbalo blanco. Las larvas se alimentaron con rotíferos *Brachionus plicatilis* (10 a 15 mL^{-1}); nauplios de *A. tonsa* (0,25 a 0,5 mL^{-1}) y rotíferos (5 a 7,5 mL^{-1}), y nauplios de *A. tonsa* (0,12 a 0,25 mL^{-1}). El promedio de supervivencia de los tratamientos fue 11,9%. A los 20 días de edad, las larvas fueron sometidas a estrés térmico. Posteriormente, se evaluó la resistencia al estrés. Las larvas de róbalo blanco alimentadas con *B. plicatilis*+*A. tonsa* alcanzaron un mayor peso y longitud (7,5 ± 0,00 mg y 9,1 ± 0,23 mm, respectivamente) y resistieron más al estrés por calor (87,4%) que las larvas alimentadas con los demás alimentos, lo que indica que la mezcla de alimentación es satisfactoria como una dieta inicial para larvas de róbalo blanco. Sin embargo, se necesita más investigación para confirmar estos resultados.

Palabras clave: crustáceos, róbalo blanco, larvicultura, alimento vivo, peces marinos, acuicultura.

INTRODUCTION

Studies on the common snook *Centropomus undecimalis* larvae have been conducted primarily in the United States, Mexico and Brazil, with eggs obtained from wild breeding mature specimens and recently by Mote Marine Laboratory using fish in captivity (Yanes-Roca & Main, 2012).

The results of *Centropomus* spp. larviculture in Brazil are described for the fat snook, *C. parallelus*.

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However, in Brazil, for the common snook, this is the first result of experiments with larvae obtained from captive breeding as the only work done previously with larvae of the common snook was from spawning wild breeding specimens (Soligo *et al.*, 2011).

One important factor in the production of juvenile marine fish is the live food supply during incubation, because they stimulate food intake and secretion of enzymes, resulting in continued growth and good survival (Chang *et al.*, 2006).

Marine copepods are sources of protein, lipids [especially the highly unsaturated fatty acids eicosapentaenoic acid (EPA) 20:5 n-3 and docosahexaenoic acid (DHA) 22:6 n-3], carbohydrates, and enzymes which are essential for the survival, growth and digestion; metamorphosis of larvae developing central nervous system, maintaining the structure and function of the cell membrane and the development and operation of the vision and stress tolerance (Sargent *et al.*, 1997; Støttrup, 2000) and these advantages are important as live food for growing fish.

Acartia tonsa is one of the most studied species of copepods. Their nauplii are between 65 and 120 mm wide and 106 to 250 mm length and can be fully digested by fish larvae (Schipp *et al.*, 1999). They are used in farmed fish because they are effective in the first feeding (Schipp *et al.*, 1999), as when fed with a mixture of microalgae, these copepods are an excellent source of highly unsaturated fatty acids in the polar lipid fraction, which are biologically available to the larvae and are a source of antioxidants, astaxanthin and vitamins C and E (Schipp *et al.*, 1999). The ability to incorporate essential fatty acids for marine fish larvae through their phytoplankton diet may be the answer to the success of copepods as live food (Sargent *et al.*, 1997; Støttrup, 2000). Arachidonic acid (ARA) and EPA-derived eicosanoids are involved in the physiological response to stress and, probably, the optimum ratio of EPA: ARA found in copepods allows fish larvae to cope better with stressful situations (McEvoy & Sargent, 1998).

In the larviculture of genus *Centropomus* spp., the survival rate is generally low, and a higher incidence of deaths occurs in the first week because of the difficulty of adapting to the first food (Yanes-Roca & Main, 2012). Successful larval rearing in the early days is a key for the production of fish species of commercial importance (Cara *et al.*, 2005).

Quantifiable indicators of stress have been sought by farmers to monitor the impact of the conditions and management in hatcheries (Cara *et al.*, 2005) and different stress resistance tests are used for this purpose: the confinement (Arends *et al.*, 1999), temperature variations and exposure to low levels of oxygen (Tago *et al.*, 1999), osmotic shock (Van Anholt *et al.*, 2004), exposure to air (Van Anholt *et al.*, 2004; Luz & Portella, 2005) used in both the larval fish in freshwater and saltwater/brackish.

The initial diet of common snook larvae is not well defined. Several studies have shown that inclusion of copepods in the initial larval diet of the common snook and fat snook is positive (Barroso *et al.*, 2013; Yanes-Roca & Main, 2012); however, it is still necessary to prove that copepods increase resistance to stress, to improve the survival and growth when producing captive specimens. Therefore, the aim of this study was to evaluate if the thermal stress resistance of *C. undecimalis* larvae increases by introducing copepod *A. tonsa* nauplii in their food.

MATERIALS AND METHODS

This study was conducted at the Laboratório de Piscicultura Marinha (LAPMAR), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC. The experiment was approved by the Ethics Committee on the Use of Animals/UFSC (Protocol PP00861).

The common snook broodstock were kept in circular tanks of 36,000 L in a water recirculation system. To induce spawning, we chose two males that had a fluidity of sperm and one female that had oocytes with an average of 350 µm based on the study of Ibarra-Castro *et al.* (2011). The eggs were quantified by a volumetric method and transferred to fiberglass tanks with a 100-L useful volume at a density of 15 eggs L^{-1}. The hatching rate was 98%.

The rotifer *B. plicatilis* (average size of 120 to 300 µm) was cultured in seawater salinity 35, average temperature of 26°C and fed once a day with algae *Nannochloropsis oculata* ($300x10^4$ cells mL^{-1}) and baker's yeast *Saccharomyces cerevisiae* (0.8 g 10^6 rotifers, divided into three parts, which are offered at 9, 13 and 17 h). Before offering larvae, rotifers were enriched with a commercial emulsion Protein Selco® Plus, INVE, Belgium (150 g m^{-3} for 12 h), to improve the nutritional quality.

Broodstock *A. tonsa* were obtained from a pond filled with water of Lagoa da Conceição (Florianópolis-SC) that was filtered through a 100 µm mesh in a system of air lift (Barroso *et al.*, 2013). Copepods were isolated, identified and cultured in the laboratory in fiberglass tanks with 250 L of seawater salinity 35, an average temperature of 28°C, to obtain the nauplii, with modified methods (Støttrup *et al.*, 1986). The feeding of the copepods was conducted with three species of

microalgae in the exponential phase of growth: *Chaetoceros calcitrans*, *Isochrysis galbana* and *N. oculata* (500; 400 and 300x10^4 cells mL^{-1}, respectively), which are microalgae that contain essential fatty acids for marine fish larvae.

Larviculture took place in the green water system, to which microalgae *N. oculata* were added daily in the tanks while maintaining a density of 500x10^4 cells mL^{-1} in fiberglass tanks, circular, with a working volume of 100 L. The renewal of the water of the experimental units began at 9 days, with 50% until day 13, when they went to 100% at the end of the experiment.

The larvae were fed from 2-days-old until 19 with three different diets: 1) rotifers *B. plicatilis* density 10 to 15 rotifers mL^{-1}; 2) *A. tonsa* copepod nauplii, density between 0.25 and 0.5 nauplii mL^{-1}; and 3) rotifers (5 to 7.5 mL^{-1}) + *A. tonsa* nauplii (0.12 to 0.25 mL^{-1}), half of the density of each regime. Table 1 contains the densities and periods in which larvae were offered. Rotifers were offered to the larvae on the second day after hatching at the same density used by Ibarra-Castro *et al.* (2011).

The number of nauplii and rotifers was measured once a day, in the morning, to keep the determined density for each experimental treatment in the hatchery tanks. With the help of a 100-mL flask, a water sample was taken from each tank; a sub-sample of 1 mL and lugol was examined under a microscope to count the number of organisms.

Temperature (27 ± 1ºC), salinity (34) and dissolved oxygen (7.3 ± 0.7 mg L^{-1}) remained controlled during the experimental period and at levels considered optimal for the species (Yanes-Roca & Main, 2012). A photoperiod of 10 h light: 14 h dark was maintained.

At the end of the experiment, the wet weight (mg) of 24 larvae was measured with a precision balance. The total length (mm), the percentage of larvae with gas bladder and the notochord flexion of larvae was measured directly with the aid of a stereomicroscope.

The stress test by heat shock was carried out on 20-day-old larvae. Before the test, common snook larvae went through a period of food deprivation for 3 h, in containers of 5 L of sea water, with the same conditions of salinity, dissolved oxygen and temperature in which they were maintained in a 100-L tank hatchery.

Twenty-nine larvae from each treatment in triplicate were carefully removed from each tank, with the aid of a 500-mL vessel. The larvae were then placed in a 5-L vessel (containing the same water in which they were in before the temperature of 27°C) with a sieve therein to be transferred to a vessel containing seawater at 37°C. Acute heat shock (27 to 37°C) lasted 10 min. Then, larvae were carefully returned to their original containers at 27ºC. Twenty-four hours later the survival was evaluated for the definition of stress resistance rate (Re), where (Re) = [(number of live larvae in the container / total number of larvae in the container)] x 100 (Ako *et al.*, 1994).

Data of wet weight (mg) and length (mm) were tested for normality (Shapiro-Wilk test) and homoscedasticity (Levene test) before being analyzed by ANOVA. Then, when necessary, the means were compared by Tukey's test. The data of the rate of inflation of the gas bladder and flexion notochord were analyzed by ANOVA.

We used the nonparametric χ^2 for comparison of survival (%) after stress test between treatments. The first test was with a 3x2 contingency table and notice significant differences, 2x2 tables were used to find the differences. All analyses were performed using $\alpha = 0.05$.

RESULTS

The common snook larval survival reared with different foods was greater with a mix of rotifers *B. plicatilis* + *A. tonsa* nauplii (13.7%) and showed higher wet weights (mg) and lengths (mm) than other treatment (Table 2). All larvae in the study flexed their notochords (Table 2) between 10 and 12 days of age.

The larvae fed a mixture endured more stress after heat shock (Table 3).

Table 1. *Brachionus plicatilis* and *Acartia tonsa* nauplii densities and periods of feeding *Centropomus undecimalis* larvae.

Treatment	Live feed	Density (mL^{-1})	Period (days)
Rotifer	*B. plicatilis*	10.00	2º to 10º
		15.00	11º to 19º
Nauplii of copepod	*A. tonsa*	0.25	2º to 6º
		0.50	7º to 19º
Mix	*B. plicatilis*	5.00	2º to 10º
Mix	*A. tonsa*	0.12	2º to 6º
Mix	*B. plicatilis*	7.50	11º to 19º
Mix	*A. tonsa*	0.25	7º to 19º

Table 2. Survival (S) (%), wet weight (WW) (mg), total length (TL) (mm), inflated gas bladder (IGB) (%) and flexion of the notochord (FN) (%) in common snook *Centropomus undecimalis* at 19 days, fed rotifers *Brachionus plicatilis* (Bp); *Acartia tonsa* nauplii (At) and a mixture of them (Bp + At). n = 24. Mean and standard deviation (±SD). Different superscript letters in the same column indicate significant difference ($P < 0.05$).

Treatments	S	WW	TL	IGB	FN
Bp	10.8	$1.4^{c} \pm 0.00$	$5.3^{c} \pm 0.09$	$79.17^{ab} \pm 0.00$	100 ± 0.00
At	11.2	$4.4^{b} \pm 0.00$	$7.7^{b} \pm 0.10$	$95.83^{a} \pm 0.00$	100 ± 0.00
Bp + At	13.7	$7.5^{a} \pm 0.00$	$9.1^{a} \pm 0.23$	$70.83^{b} \pm 0.00$	100 ± 0.00

Table 3. Survival (%) of 20-day-old *Centropomus undecimalis* larvae 24 h after heat shock fed *Brachionus plicatilis* rotifers, *Acartia tonsa* nauplii and a mixture of them (n = 3). Mean and standard deviation (±SD). Different superscript letters in the same column indicate significant difference ($P < 0.05$).

Treatments	Survival
B. plicatilis rotifer	$4.60^{c} \pm 2.31$
A. tonsa nauplii	$43.68^{b} \pm 5.69$
Rotifer + *A. tonsa* nauplii	$87.36^{a} \pm 1.15$

DISCUSSION

Barroso *et al.* (2013), comparing the same types of food utilized in this study in the newly hatched larvae fat snook, *Centropomus parallelus*, found an average survival rate of 16.0%, with 14-day-old, and claim that this is a tendency for other marine fish species. This trend is explained by the fact that in the early days old larvae of marine fish are very fragile, with low survival, as the energy demand and protein necessary for morphological changes such as formation of the mouth, anus, pigmentation of the eyes, gas bladder, fins, scales and other organs of the digestive system is very large (Yúfera & Darias, 2007).

Epinephelus coioides larvae fed rotifers and copepod nauplii (0.1 nauplii mL^{-1}) increased their growth and survival (Knuckey *et al.*, 2005), while the larvae of *C. parallelus* fed a mixture of rotifers + *A. tonsa* nauplii did not differ significantly from larvae fed rotifers or *A. tonsa* nauplii, reaching an average of 3.86 mm at 14 days (Barroso *et al.*, 2013). In the present study, common snook larvae fed mix rotifers + *A. tonsa* nauplii showed higher wet weights (mg) and lengths (mm) than other treatments.

In describing the development of the larvae of *C. undecimalis*, reared in the laboratory, Lau & Shafland (1982) found a total length of 9.5 mm. These authors fed larvae for the first 12 days of age with natural zooplankton (mainly copepods nauplii) and rotifers, and then with newly hatched *Artemia* sp. In the present study, larvae fed a mixture of rotifers + *A. tonsa* nauplii until 19 days old, reached growth levels of larvae also fed *Artemia* sp. (Lau & Shafland, 1982). This suggests an alternative to the use of *Artemia* for the larval period studied, since from the point of view of nutrition, a diet based on rotifers and *Artemia* can be completely replaced or supplemented with the use of copepods compared to the fatty acid profile of the composition of these animals, which meets the needs of the larval fish (Sargent *et al.*, 1997).

Gas bladder formation allows to vertically displacing the larvae in the water column while the bending of the notochord is a prerequisite for the formation of the fin, which is important in swimming horizontally (Barroso *et al.*, 2013). Although mixed treatment larvae had a higher growth, higher gas bladder inflation was seen in the treatment with nauplii larvae of *A. tonsa*. This can be explained because the other treatments had enriched rotifers, when they are offered to the larvae take with them the enriching which is mainly composed of fatty acids which may form a layer on the surface of the water making it difficult to capture the air larvae at the time of inflating gas vesicle. Larvae that do not inflate their gas bladders are less resistant to stresses such as handling, hypoxia and weaning (Chatain, 1989), but the larvae fed a mixture endured more stress after heat shock, because stress resistance is also related to the quality of the food (Luz, 2007) and in this study, the mixture is described as an option ensures greater resistance.

Osteological development studies of *C. undecimalis* larvae, grown in the laboratory, found that the bending of the notochord occurred between 10 and 14 days old and 4.4 mm (Potthof & Tellock, 1993). In the present study all larvae flexed their notochords between 10 and 12 days of age.

Heat shock at 10°C applied to the common snook larvae showed significant differences depending on the type of food ($P < 0.05$) and resistance to stress after 24 h. The larvae fed nauplii of *A. tonsa* were more resistant than larvae fed rotifer only. According to Watanabe *et al.* (1983), malnourished fish do not survive in extreme conditions compared to properly fed fish. In the

intensive hatchery, animal stress is constant (Luz, 2007) and feeding interferes in larval resistance to stress (Luz, 2007), as verified by Ako *et al.* (1994), when it increased the amount of fatty acids in *Artemia* to feed the larvae of *Mugil cephalus* and realized that they became more resistant to stress responses.

The advantages of copepods relative to other organisms as feed have been found in several studies. These results clarify the benefits observed with regard to resistance to heat stress and common snook larvae growth. The stress response may represent an important tool for selecting the best organisms for aquaculture, especially those raised in intensive systems (Lima *et al.*, 2006). Furthermore, it has been shown that organisms, which have induced thermotolerance, also show increased resistance to other forms of stress (Spees *et al.*, 2002).

In this study, we conclude that the mix of rotifers and copepods of *Acartia tonsa* nauplii provided greater common snook larval growth and increased resistance to heat stress. However, more research is needed to confirm these results.

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