FIRST FEEDING OF *Eugerres brasilianus* (CARAPEVA) LARVAE WITH *Acartia tonsa* (COPEPOD) NAUPLII INCREASES SURVIVAL AND RESISTANCE TO ACUTE STRESS[1]

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ABSTRACT: The rotifer *Brachionus* sp. is commonly used for larval feeding in marine fish hatcheries. The aim of this study was to evaluate whether the inclusion of *Acartia tonsa* nauplii in the initial diet of carapeva larvae improves their survival, growth and resistance to stress when compared to the regimen containing only rotifers. Adult copepods were collected in the wild and cultured with the microalgae *Chaetoceros muelleri*, *Isochrysis galbana* and *Nannochloropsis oculata* to obtain nauplii. Carapeva larvae were grown for 15 days using four treatments and three replicates: 1) *Brachionus plicatilis* rotifers (10 to 15/mL); 2) *A. tonsa* nauplii (0.25 to 0.5/mL); 3) *Brachionus plicatilis* rotifers (5 to 7.5/mL) + *A. tonsa* nauplii (0.12 to 0.25/mL), and 4) no supply of live feed. After 15 days, the carapeva larvae were subjected to stress by exposure to air for 10 seconds and then returned to the source tank to evaluate survival after 24 h. Survival and stress resistance were higher in carapeva larvae fed *B. plicatilis* + *A. tonsa* nauplii ($P<0.05$), 20.9 ± 11.2% and 88.9%, respectively. These results confirm the positive effect of the inclusion of copepod nauplii in the diet of fish larvae. However, more research is needed to validate these results.

Keywords: hatchery, live feed, marine fish, stress resistance.

ALIMENTAÇÃO INICIAL DE LARVAS DE CARAPEVA Eugerres brasilianus COM NÁUPLIOS DE COPÉPODO Acartia tonsa MELHORA A SOBREVIVÊNCIA E RESISTÊNCIA AO ESTRESSE AGUDO

RESUMO: O rotífero *Brachionus* sp. é o alimento vivo mais comumente utilizado na larvicultura de peixes marinhos. O objetivo deste estudo foi avaliar se a inclusão de náuplios de *Acartia tonsa* na alimentação inicial de larvas de carapeva melhora sua sobrevivência, crescimento e resistência ao estresse, comparado com o regime contendo apenas rotíferos. Copépodos adultos foram coletados no ambiente e cultivados com as microalgas *Chaetoceros muelleri*, *Isochrysis galbana* and *Nannochloropsis oculata* para obtenção dos náuplios. As larvas de carapeva foram cultivadas por 15 dias com quatro tratamentos e três repetições: 1) rotíferos *Brachionus plicatilis* (10 a 15/mL); 2) náuplios de *A. tonsa* (0,25 a 0,5/mL); 3) rotíferos *Brachionus plicatilis* (5 a 7,5/ mL) + náuplios de *A. tonsa* (0,12 a 0,25/mL) e 4) sem suplementação de alimento vivo. Após 15 dias, as larvas de carapeva foram submetidas a estresse por exposição ao ar, por 10 segundos e retornadas aos tanques para avaliar a sobrevivência após 24 h. As larvas alimentadas com rotífero *B. plicatilis* + náuplio de *A. tonsa* apresentaram maiores sobrevivência e resistência ao estresse ($P<0,05$), 20,9 ± 11,2% e 88,9%, respectivamente. Esses resultados confirmam o efeito positivo da inclusão de náuplios de copépodos na dieta de larvas de peixes. Contudo, são necessárias mais pesquisas para validar esses resultados.

Palavras-chave: alimento vivo, larvicultura, peixe marinho, resistência ao estresse.

INTRODUCTION

There are several native Brazilian marine fish species, such as carapeva, with a potential for rearing. However, there is a lack of information on the biology and farming technology of these species (Cavalli and Hamilton, 2009). Common throughout the Brazilian coast, *Eugerres brasilianus* (Cuvier, 1830), popularly known as carapeva or caratinga, is a member of the Gerreidae family, which can reach up to 40 cm in length (Figueiredo and Menezes, 1980). This species plays an important role in commercial, artisanal and sport fishing (Bezerra *et al*., 2001).

Copepods can be an alternative source for feeding marine fish larvae (Olivotto *et al*., 2008), since they are an effective feed for commercially important fish species as they contain macro- and micronutrients, lipids, especially highly unsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid), protein, carbohydrates, and enzymes. These compounds are essential for the survival, growth, digestion, larval metamorphosis, development of the central nervous system, maintenance of cell membrane structure and function, development of sight, and stress tolerance of fish (Bromage and Roberts, 1995; Sargent *et al*., 1997; Schipp *et al*., 1999; Støttrup, 2000).

Copepod nauplii of the order Calanoida contain the complete profile of nutrients required for the growth and survival of marine fish larvae (Stottrup, 2000; Drillet *et al*., 2007) and have an ideal size (average of 65 µm) to be used for the first feeding of very small fish larvae (Schipp *et al*., 1999). The calanoid *Acartia tonsa* is used as feed for fish larvae such as *Gadus morhua*, *Lutjanus johnii* and *L. argentimaculatus* (Stottrup and McEvoy, 2003), *Centropomus parallelus* (Barroso *et al*., 2013), and *C. undecimalis* (Yanes-Roca *et al*., 2013).

In general, studies investigating the feeding efficiency of fish larvae only consider growth and survival. In some cases, the physiological state of the larvae is assessed using stress tests. During larval rearing, performance variables, growth and survival are the main indicators of food quality management (Luz *et al*., 2012). In addition to these variables, testing the resistance to stress can be a complementary tool to evaluate the quality of fry and fingerling production and to establish the effects of diet on improving animal health (Ako *et al*., 1994; Luz, 2007).

The exposure of larvae to air for a predetermined period of time is a commonly used stress test (Benfey and Biron, 2000; Martins *et al*., 2000; Koven *et al*., 2001; van Anholt *et al*., 2004, Luz and Portella, 2005; Luz, 2007) and is effective in assessing the effect of food quality on the rate of stress resistance (Ako *et al*., 1994; Kanazawa, 1997; Luz, 2007; Luz *et al*., 2012).

Therefore, the aim of this study was to determine whether the inclusion of *A. tonsa* nauplii in the initial diet of carapeva larvae improves their growth, survival, and stress resistance.

MATERIALS AND METHODS

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

Maintenance and spawning induction of broodstock carapeva

Broodstock of carapeva were kept in 2 x 2 x 1.5 m cages in the nursery at an average water salinity of 25. For spawning induction, broodstocks (2 males:1 female) were chosen among those exhibiting semen fluidity and oocytes with an average size of 350 µm. Spawning was induced with a dose of 15 mg/kg of luteinizing hormone releasing hormone analog (Passini *et al*., 2013) and broodstocks were kept in 500-L tanks containing circulating pond water until spawning. After spawning, the eggs were collected through the water outlet of the broodstocks tank into a cylindrical fiberglass incubator (capacity of up to 6 L). Embryonated eggs were transferred to circular fiberglass tanks with a working volume of 100 L at a density of 10 eggs/L for larval testing.

Live feed

The rotifer *Brachionus plicatilis* (average length of 120-300 µm) was cultured in 600-L tanks and fed once a day the microalga *Nannochloropsis oculata* (300×10^4 cells/mL) and *Saccharomyces cerevisiae* (0.8 g/10^6 rotifers, divided into three portions offered throughout the day). Before administration to the larvae, the rotifers were enriched with a commercial diet (Protein Plus Selco®, INVE, Belgium; 150 g/m^3) for 12 h.

Acartia tonsa adults were obtained from a nursery consisting of water from Lagoa da Conceição (Florianópolis, SC, Brazil) by filtering through a 200-µm mesh. Adult copepods were isolated, identified and cultured in a fiberglass tank containing 250 L

of seawater with a salinity of 35 and temperature of 28°C to obtain nauplii according to a method modified from Stottrup *et al.* (1986). The feeding was carried out with three microalgal species in the exponential growth phase: *Chaetoceros calcitrans*, *Isochrysis galbana* and *N. oculata* (500 x 10^4, 400 x 10^4 and 300 x 10^4 cells/mL, respectively). The average size of the nauplii during culture ranged from 63 to 67 µm.

Hatchery

Carapeva larvae were grown from day 2 to day 15 of age in a completely randomized design consisting of four different food regimens and three replicates: 1) *B. plicatilis* rotifers (10 to 15/mL); 2) *A. tonsa* nauplii (0.25 to 0.5/mL); 3) *B. plicatilis* rotifers (5 to 7.5/ mL) + *A. tonsa* nauplii (0.12 to 0.25/mL) whose densities are shown in Table 1, and 4) no supply of live feed.

The density of nauplii and rotifers was determined once a day in the morning. For this purpose, a 100-mL sample was collected from each water tank, and a subsample of 1 mL was fixed with Lugol and observed under a stereoscopic microscope. The larvae were reared in a green water system, adding *N. oculata* at a density of 50 x 10^4 cells/mL.

The variables salinity (34 ± 2), temperature (26.8 ± 1.5°C) and dissolved oxygen (6.2 ± 1.8 mg/L) were measured daily using a refractometer (Atago, S10-E) and a multiparameter probe (Alfakit) and were within acceptable levels for *E. brasilianus* larvae (Alvarez-Lajonchère *et al.*, 1996). On day 15 of the experiment, the larvae were counted to calculate survival and the total length (mm) of a sample of 30 larvae per replicate was measured, except for the treatment without food in which only 16 larvae survived.

Stress test

A sample of 36 larvae per replicate, separated at random, were kept for 2 h in containers with 5 L of clean seawater, in starvation, under the same conditions of temperature and salinity of the hatchery. Next, the larvae were subjected to an acute stress test by exposing them to air on absorbent paper for 10 s using a method adapted from Luz (2007). The duration was determined in previous tests, which demonstrated the absence of survivors after this period. After the test, the larvae were returned to 5-L containers and survivors were counted 24 h later to determine the rate of stress resistance defined as the percentage of survival (Ako *et al.*, 1994).

Statistical analysis

The survival data (%) and total length (mm) were analyzed for normality (Shapiro-Wilks test) and homoscedasticity (Levene) and then subjected to analysis of variance. Means were compared by Tukey's test. The nonparametric χ^2 test was applied for comparison of post-stress survival (%) between treatments. The first test was performed to compare the three treatments (1, 2 and 3) supplying live feed (3x2 contingency table) and finding significant differences 2x2 contingency table was used to find the differences.

Table 1. Densities of *Brachionus plicatilis* and *Acartia tonsa* nauplii and periods of feeding *Eugerres brasilianus* larvae

Treatment	Live feed	Density (organisms/mL)	Period (days)
Brachionus plicatilis rotifers	*B. plicatilis*	10	2 to 10
	B. plicatilis	15	11 to 15
Acartia tonsa nauplii	*A. tonsa*	0.25	2 to 6
	A. tonsa	0.50	7 to 15
Brachionus plicatilis rotifers + *A. tonsa* nauplii	*B. plicatilis*	5	2 to 10
	A. tonsa	0.12	2 to 6
	B. plicatilis	7.5	11 to 15
	A. tonsa	0.25	7 to 15

RESULTS

The highest survival of carapeva larvae was observed for the combination of *B. plicatilis* rotifers (5 to 7.5/mL) + *A. tonsa* nauplii (0.12 to 0.25/mL) ($P<0.05$) and the lowest survival for the treatment that did not supply live feed (Table 2). The total length of the larvae not receiving live feed was significantly lower than that observed for the other treatments ($P<0.05$) (Table 2), with an average length of 5.61 mm.

The larvae fed *B. plicatilis* rotifers (5 to 7.5/mL) + *A. tonsa* nauplii (0.12 to 0.25/mL) were more resistant ($P<0.05$) than the larvae submitted to the other treatments (Table 3). The *B. plicatilis* rotifers (10 to 15/mL) and *A. tonsa* nauplii (0.25 to 0.5/mL) treatments did not differ significantly and showed low rates of resistance in the stress test (Table 3). However, differences were found between larvae fed the combination and the other treatments. The lowest rate of resistance was observed for the starvation treatment.

DISCUSSION

The inclusion of *A. tonsa* nauplii in the diet of carapeva larvae exerted an important effect on survival. The combination of *B. plicatilis* rotifers (5 to 7.5/mL) + *A. tonsa* nauplii (0.12 to 0.25/mL) provided results similar to the exclusive treatment with *B. plicatilis* rotifers, while survival was lower for the diet containing only *A. tonsa* nauplii. On the other hand, no significant differences in length gain were observed. It is likely that in the treatment with *A. tonsa* nauplii, with fewer surviving larvae, the number of nauplii available for each larva was not sufficient to impair growth.

On the other hand, the observation of larvae surviving in the absence of live feed was not expected. It is possible that microorganisms (bacteria and protozoa, among others) present in the water tank and on surfaces (biofilm) were used as feed. THOMPSON *et al*. (1999) observed that microorganisms could be a food source because of their high concentrations of N and P, allowing the survival of *Penaeus paulensis* larvae for long periods. Furthermore, ideal conditions of factors such as temperature and salinity can influence the ability of larvae to survive starvation (MCGURK, 1984).

From a nutrition point of view, a diet containing rotifers and *Artemia* can be fully replaced or supplemented with copepods because their profile of fatty acids and other nutrients meet the

Table 2. Mean and standard deviation of the survival and total length of carapeva larvae according to treatment

Treatment	Survival (%)	Total length (mm)	N
Brachionus plicatilis rotifers	16.8 ab ± 10.7	5.12 a ± 0.08	30
Acartia tonsa nauplii	7.8 b ± 1.5	5.88 a ± 0.06	30
Brachionus plicatilis rotifers + *A. tonsa* nauplii	20.9 a ± 11.2	5.83 a ± 0.38	30
No supply of live feed	3.2 b ± 4.8	2.64 b ± 2.30	16

Means in the same column followed by different lowercase letters differ significantly ($P<0.05$).

Table 3. Survival (%) of 15-day-old carapeva larvae after the stress test according to treatment

Treatments	Survival after stress
Brachionus plicatilis rotifers	55.56 b ± 1.00
Acartia tonsa nauplii	52.78 b ± 2.65
Brachionus plicatilis rotifers + *A. tonsa* nauplii	88.89 a ± 2.00
No supply of live feed	10.28 c ± 3.51

Means in the same column followed by different lowercase letters differ significantly ($P<0.05$).

requirements of marine fish larvae (SARGENT *et al.*, 1997; MCKINNON *et al.*, 2003). In the present study, complete replacement with *A. tonsa* nauplii was not satisfactory because it compromised survival. The limiting factor was probably the amount offered to the larvae, not its quality. In previous studies on carapeva larvae (initial density of 1 to 10 larvae/L), *Oithona* sp. nauplii were offered at densities of 0.5 to 2.0/mL (ALVAREZ-LAJONCHÈRE *et al.*, 1996; HERNÁNDEZ-MOLEJÓN and ALVAREZ-LAJONCHÈRE, 2003), a density slightly higher than that used in the present study. In a study investigating carapeva larvae until 13 days of life (HERNÁNDEZ-ALVAREZ and MOLEJON-LAJONCHÈRE, 2003), the survival rates obtained with rotifers and nauplii of the copepod *Oithona oculata* were 6% and 12.5%, respectively, while no significant difference in survival was observed in the present study (16.8% and 7.8%, respectively). These differences can certainly be explained by the different environmental conditions of the two studies. However, both studies show why the initial culture phase of pelagic larvae of marine fish deserves special attention since initial mortality is very high.

The effects of copepod feeding were more evident in the case other marine fish species. For example, the inclusion of nauplii of the calanoid *Gladioferens imparipes* in combination with rotifers for the first feeding of *Glaucosoma hebraicum* and *Pagrus auratus* larvae increased survival and growth (PAYNE *et al.*, 2001). Fat snook (*Centropomus parallelus*) larvae receiving a diet that contained the copepod *A. tonsa* had higher survival, growth, and essential fatty acid levels than those fed a diet containing only rotifers (BARROSO *et al.*, 2013). These results highlight the importance of supplementation of the diet of marine fish larvae with copepod nauplii because they are small enough to allow their capture (SU *et al.*, 2005) and do not require prior enrichment with lipid emulsions, as do rotifers and meta-nauplii of *Artemia franciscana* (LAVENS and SORGELOOS, 1996; KNUCKEY *et al.*, 2005), which are deficient in highly unsaturated fatty acids. The total lipid composition of copepod nauplii contains on average 40% docosahexaenoic acid and 16% eicosapentaenoic acid, amounts that appear to meet the nutritional requirements of marine fish larvae (VAN DER MEEREN *et al.*, 2008).

In the present study, another important effect of including *A. tonsa* nauplii in the diet of carapeva larvae was clearly observed in the stress test. The combination of *B. plicatilis* rotifers (5 to 7.5/mL) + *A. tonsa* nauplii (0.12 to 0.25/mL) provided the highest resistance to acute stress, which was higher than that obtained with the individual diets. This superiority of the copepod compared to the rotifer in terms of stress tolerance has also been demonstrated for pompano (*Trachinotus carolinus*) larvae fed *Pseudodiaptomus pelagicus* nauplii, and is related to the better fatty acid composition found in nauplii (CASSIANO *et al.*, 2012). In the case of mahi-mahi (*Coryphaena hippurus*) larvae, the highest stress resistance was observed when high concentrations of docosahexaenoic acid were present in the diet, i.e., copepod or *Artemia* enriched with fatty acids (KRAUL *et al.*, 1993).

The culture of pelagic copepods with a short life cycle and rapid growth using semi-intensive high-throughput technologies has been proposed for tropical and subtropical countries. This approach may have economic benefits, providing highly stress resistant larvae with good survival and growth (HERNÁNDEZ-ALVAREZ and MOLEJÓN-LAJONCHÈRE, 2003). The stress response is a very important tool to select the best individuals for aquaculture, especially those raised in intensive systems (LIMA *et al.*, 2006). In this respect, the results of this study indicate that continuation of the use of hatchery diets that promote a survival rate of only slightly over 50% in the stress test is inappropriate, considering that this phase is already critical and survival is low.

In conclusion, the resistance of carapeva larvae to acute stress (air exposure) was higher when the diet was supplemented with *B. plicatilis* rotifers + *A. tonsa* nauplii. We emphasize that it is still necessary to refine the use of nauplii as part of a first-feeding scheme due to the difficulties in producing nauplii on a large scale.

ACKNOWLEDGEMENT

The authors thank CAPES for the fellowship granted to the first author within the Programa Amazônia Azul, and CNPq for granting a research fellowship to Professor Vinicius Cerqueira and fellowships to the third and fifth authors. We thank Professor Mauro de Melo Júnior for his help with the identification of the copepods used in this study. We are also grateful to the LAPMAR technicians and students for help with the logistics of fish reproduction.

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