



Monoculture of the ciliate protozoan *Euplotes* sp. (Ciliophora; Hypotrichia) fed with different diets

Guilherme de Freitas Côrtes, Monica Yumi Tsuzuki* and Emilio Mateus Costa Melo

Centro de Ciências Agrárias, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rod. Admar Gonzaga, 1346, 88034-001, Itacorubi, Florianópolis, Santa Catarina, Brazil. *Author for correspondence. Email: mtsuzuki@cca.ufsc.br

ABSTRACT. Ciliate protozoa of the genus *Euplotes* commonly appears contaminating mass cultures of rotifers but also with potential to be used as live food in the larviculture of marine fish. To obtain a monoculture of *Euplotes* sp., three diets were tested: 1) microalgae *Nannochloropsis oculata*, 2) commercial diet for rotifers Culture Selco 3000, and 3) baker's yeast (*Saccharomyces cerevisiae*). The ciliates were inoculated at 10 ind. mL⁻¹. On day 5, protozoa densities in the groups fed the commercial diet (1,911.0 ± 248.7 ind. mL⁻¹) and the baker's yeast (2,600.0 ± 195.3 ind. mL⁻¹) did not differ, but were higher than the group fed microalgae (2.0 ± 1.4 ind. mL⁻¹) (p < 0.05). On day 6, the density in the group fed baker's yeast was higher (15,484.0 ± 1,164.9 ind. mL⁻¹) than in the groups fed microalgae (3.0 ± 1.4 ind. mL⁻¹) or commercial diet (11,287.0 ± 1,468.0 ind. mL⁻¹). An exponential growth curve was observed for the protozoa fed baker's yeast (R² = 0.992; p < 0.05) and commercial diet (R² = 0.979; p < 0.05). The microalgae diet did not result in satisfactory growth of the protozoan. Feeding baker's yeast or commercial rotifer's diet to a monoculture of *Euplotes* sp. can result in very high densities of this protozoan.

Keywords: live food, production, microalgae, rotifers, larviculture, marine fish.

Monocultivo do protozoário ciliado *Euplotes* sp. (Ciliophora; Hypotrichia) com diferentes dietas

RESUMO. Protozoários ciliados do gênero *Euplotes* comumente aparecem como contaminante em culturas massivas de rotíferos, apresentando potencial para ser utilizado na larvicultura de peixes marinhos. Para obter um monocultivo de *Euplotes* sp., foram testados três tipos de alimento: 1) microalga *Nannochloropsis oculata*, 2) dieta comercial para rotíferos Culture Selco 3000, e 3) fermento biológico (*Saccharomyces cerevisiae*). Os ciliados foram inoculados na concentração de 10 indivíduos mL⁻¹. No quinto dia, a densidade de protozoários nos tratamentos com dieta comercial para rotíferos (1.911,0 ± 248,7 ind. mL⁻¹; média ± desvio padrão) e fermento biológico (2.600,0 ± 195,3) não diferiram entre si, mas foram superiores ao tratamento com microalga (2,0 ± 1,4) (p < 0,05). Ao sexto dia de cultivo, a densidade no tratamento com fermento (15.484,0 ± 1.164,9 ind. mL⁻¹) foi superior aos tratamentos com microalga (3,0 ± 1,4 ind. mL⁻¹) e dieta comercial (11.287,0 ± 1.468,0 ind. mL⁻¹). O cultivo destes ciliados apresentou uma curva de crescimento exponencial para o fermento (R² = 0,992; p < 0,05) e a dieta comercial (R² = 0,979; p < 0,05). O uso da microalga *N. oculata* não resultou em crescimento satisfatório destes organismos. O uso de fermento biológico e dieta comercial para rotíferos no monocultivo de *Euplotes* sp., pode resultar em altas densidades do protozoário.

Palavras-chave: alimento vivo, produção, microalga, rotíferos, larvicultura, peixes marinhos.

Introduction

Ciliate protozoa are ubiquitous in aquatic environments and one of main consumers of bacteria in eutrophic waters (KAMIYAMA, 1994). Because of their abundance and size - similar or even smaller than nauplii of copepods, probably they occupy an important position on aquatic trophic food web. According to Holt and Holt (2000), marine fish larvae in the wild commonly feed on a large array of microzooplankton, including protozoa (tintinnid and ciliates), dinoflagellates, larvae of mollusks and, mainly, eggs and nauplii of copepods. However, there are few studies on their role as

source of live food in the early stages of fishes and invertebrates (KRAUL, 2006 apud CÔRTEZ; TSUZUKI, 2012, OLIVOTTO et al., 2005; THOMPSON et al., 1999).

Food for fish larvae is usually determined by fish mouth size, prey size and fish ability to prey, which suggests that small prey with slow swimming, such as ciliate and dinoflagellate protozoa would be more suited (HUNT VON HERBING; GALLAGER, 2000, HUNT VON HERBING et al., 2001). In the majority of marine fish larvae, especially the ornamental ones, e.g. flame angelfish (*Centropyge loricula*) (Günther, 1835), barber goby (*Elacatinus*

figaro) (Sazima, Moura; Rosa, 1997), and sunrise dottyback (*Pseudochromis flavivertex*) (Rüppell, 1835), the size of the mouth is small (CÔRTEZ; TSUZUKI, 2010, KRAUL, 2006 apud CÔRTEZ; TSUZUKI, 2012, OLIVOTTO et al., 2005), thus they need small sized food, such as Super Small rotifers, copepod nauplii or ciliate protozoa such as *Euplotes* sp.

In marine fish hatchery, the type of zooplankton to be fed as a first food to fish larvae, i.e., in the transition from endogenous (yolk reserves) to exogenous feeding, is determined not only by those factors mentioned earlier but also by its nutritional quality. To master the techniques to culture prey organisms it is essential to provide constant and controlled production, especially at high densities. Rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) are widely used in marine fish hatcheries. Both organisms meet the required characteristics but they are not always the best option due to their size, nutritional quality or visual attractiveness (STÔTTRUP; MCEVOY, 2003). Feeding copepod nauplii as a first live food may be more adequate for many species. Kraul (2006 apud CÔRTEZ; TSUZUKI, 2012) reported a 10% increase in Almaco jack *Seriola rivoliana* (Valenciennes, 1833), larvae survival when feeding copepod nauplii. Some copepods are small in the naupliar phase (68 μm , *Tisbe cucumaria*, Copepoda, Harpacticoida; 65 μm , *Acartia* spp.) and the nutritional profile meets the marine fish larvae requirements (STÔTTRUP; MCEVOY, 2003). However, the drawback of this option lies on the difficulty in rearing copepods intensively under controlled conditions.

Nevertheless, Olivotto et al. (2005) reported a 15% increase in survival rates for gobiid larvae *Gobiossoma evelynae* (Böhlke; Robins, 1968) fed *Euplotes* sp. with rotifers in comparison to the combination of rotifers and brine shrimp. Unfortunately, little information is available on the culture of the ciliate protozoan. *Euplotes* sp. is usually considered undesirable contaminant in rotifer cultures (HAGIWARA et al., 2001), however, studies have been trying to demonstrate the importance and potential of these organisms as food source for the larval stages of marine fishes (FIGUEIREDO et al., 2007, OLIVOTTO et al., 2005).

As studies describing protocols to culture ciliate protozoa are scarce, this study aimed to assess the effect of different diets in the monoculture of *Euplotes* sp. and, thus, determine the diet yielding the best growth performance. Additionally, the potential to make use of *Euplotes* sp. as first food for ornamental marine fish larvae is also discussed.

Material and methods

Experimental culture conditions

The study was performed at the Laboratório de Piscicultura Marinha (LAPMAR), Santa Catarina Federal University, Florianópolis, Santa Catarina State, Brazil. The ciliate protozoan *Euplotes* sp. was isolated from a contaminated culture of rotifers *Brachionus* sp. kept at 25°C water temperature and 25 ppt salinity. First, rotifer culture water was sieved through a 45 μm mesh net, to retain rotifers, and then through an 18 μm mesh net, to retain and isolate *Euplotes* sp. (Figure 1). Protozoan mean size was $60.0 \pm 9.4 \mu\text{m}$ (mean \pm s.d.) in length and $39.0 \pm 7.4 \mu\text{m}$ in width.



Figure 1. Ciliate protozoan *Euplotes* sp. at cell division, binary fission, scale bar 10 μm . Font: Image was obtained in the site http://www.usuarios.multimania.es/ninosantamaria/subalbum_3.html, accessed in 5/10/2010, and adapted.

Diets for *Euplotes* sp. culture

Growth of *Euplotes* sp. was assessed in a six-day feeding trial with three food sources: 1) microalgae *Nannochloropsis oculata* (Hibberd, 1981) rotifer's commercial diet Culture Selco 3000 (Protein 40% , Lipid 8.5% , Ash 7% , Moisture 5% , Vit. A 500.000 Iu kg^{-1} , Vit D3 50.000 IU kg^{-1} , Vit E 3600 ppm , Vit C 4000 ppm, INVE Technologies, Belgium); and 3) baker's yeast *S. cerevisiae* (Meyen ex Hansen, 1883). Each diet was tested with four replicates.

Baker's yeast and Culture Selco were fed at a concentration of 0.5 g per million protozoa once a day. Throughout the trial, microalgae density was monitored and kept at $2.2 \pm 1.7 \times 10^6$ cells mL^{-1} . Microalgae were cultured in modified Guillard f/2

medium under constant light and aeration, harvested in the log phase (4.0×10^6 cells mL^{-1}) and supplied to the protozoan culture.

Euplotes sp. was inoculated at a concentration of 10 ind. mL^{-1} in each of the 2-L glass experimental container. Water was previously chlorinated and dechlorinated with sodium thiosulphate, and the microalgae filtered through a $5\text{-}\mu\text{m}$ mesh net. Experimental cultures were kept under 24h light, $25 \pm 1^\circ\text{C}$ (using thermostat-heaters), salinity at 30 ± 2 ppt, constant aeration, and no water renovation. Total ammonia concentration was monitored daily with a commercial test kit (Labcon, Brazil).

Cell counting

Microalgae density was determined with a Neubauer counting chamber. Protozoan density was estimated from a 1-mL sample, after fixation with 5% formalin, using a Sedgewick-Rafter counting chamber at 5 x magnification. The ciliate protozoan (*Euplotes* sp.) is not always seen in the water column as it prefers to move on the substrate and detrital particles accumulated on the bottom of the aquaria. To ensure reliable counting, each aquarium was homogenized with strong aeration prior to sampling. All counting was performed in duplicate.

Statistical analysis

To determine the diet with best growth performance during the trial, as well as the variation of total ammonia concentration, an analysis of variance was adopted with parcels divided in time. Regression analysis was used to obtain the growth curve. A 5% level of significance was adopted. Data was processed using the software Statistica (STATISTICA, 2009).

Results and discussion

Table 1 shows the increase in abundance of the ciliate protozoan *Euplotes* sp. fed the three food sources. During the first four days of culture, all groups kept the same density. On day 5, there was no significant difference between the growth of populations in groups fed Culture Selco ($1,911.0 \pm 248.7$ ind. mL^{-1}) and baker's yeast ($2,600.0 \pm 195.3$ ind. mL^{-1}), but were statistically higher ($p < 0.05$) than the group fed microalgae (2.0 ± 1.4 ind. mL^{-1}). By the end of the trial (day 6) all groups were statistically different, when the group fed baker's yeast yielded the best result ($15,484.5 \pm 1,164.9$ ind. mL^{-1}) ($p < 0.05$), followed by the group fed Culture Selco ($11,287.0 \pm 1,468.0$ ind. mL^{-1}). The group fed microalgae presented the lowest result (237.0 ± 468.7 ind. mL^{-1}). The ciliate culture presented

exponential growth curves in the baker's yeast ($R^2 = 0.992$; $p < 0.05$) and Culture Selco ($R^2 = 0.979$; $p < 0.05$) groups.

Table 1. Density of the ciliate protozoan *Euplotes* sp. fed on baker's yeast, Culture Selco, and microalgae *N. oculata* during the six-day feeding trial. Values are mean \pm standard deviation per treatment group (n = 4). Different letters indicate significant difference between means in the row ($p < 0.05$).

Days	Baker's yeast	Culture Selco	Microalgae
	Cel. mL^{-1}	Cel. mL^{-1}	Cel. mL^{-1}
6	15,484.5 \pm 1,1164.9a	11,287.7 \pm 1,468.9b	237.0 \pm 468.7c
5	2,600.5 \pm 195.3a	1,910.0 \pm 248.7a	2.0 \pm 1.0b
4	614.7 \pm 28.3	469.0 \pm 70.3	1.25 \pm 1.0
3	387.5 \pm 52.3	339.0 \pm 79.9	1.25 \pm 1.0
2	222.2 \pm 49.2	384.5 \pm 5.4	8.0 \pm 6.7
1	27.2 \pm 3.3	22.0 \pm 4.9	4.5 \pm 3.1

During the trial, total ammonia levels in the water were low until day 3 (0.25 mg L^{-1}) but from day 4, levels in groups fed baker's yeast and Culture Selco had increased. On day 6, total ammonia concentration in the baker's yeast group and Culture Selco group had increased to 4 and 3 mg L^{-1} , respectively. Total ammonia concentration in the microalgae group was lower than the others throughout the trial with values of 0.10 mg L^{-1} (Figure 2).

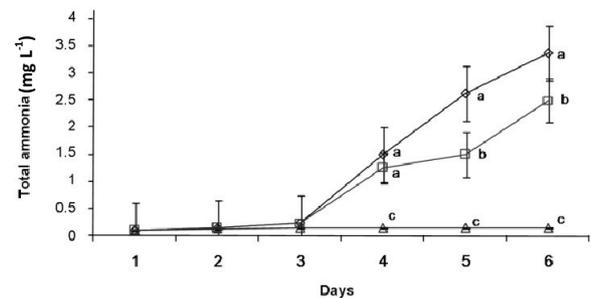


Figure 2. Total ammonia concentration (mg L^{-1}) in the water of the three test groups (baker's yeast \diamond , Selco \square , microalgae Δ) throughout the six-day feeding trial. Points in each day are mean values (n = 4), vertical bars denote standard deviation, and different letters indicate significant different between means ($p < 0.05$).

Studies have demonstrated that protozoa, including *Euplotes* sp. can feed on bacteria (WILKS; SLEIGHT, 1998). Cheng et al. (2004) reported that ciliates also feed on phytoplankton such as *N. oculata* and *Tetraselmis tetrahele* (Butcher, 1959). In the present study, the group fed the microalgae *N. oculata* did not present significant growth of the *Euplotes* sp. population. This might indicate that the concentration of microalgae was not sufficient for the protozoan growth or that the protozoan can ingest the algae but cannot digest it well. According to Cheng et al. (2004), these ciliate protozoa do not

possess a specialized jaw to grind and digest the hard cell wall of phytoplankton.

Baker's yeast was the food source resulting in the best growth but it was also the treatment with higher total ammonia concentration in the water, 4.0 mg L⁻¹. Although such relatively high concentration could inhibit growth or even kill many aquatic organisms, e.g., rotifers and fish larvae (CHENG et al., 2004; XU et al., 2004), it seems not to have affected the growth of the *Euplotes* sp. population. Xu et al. (2004) reported that total ammonia concentrations of 100 mg L⁻¹ in the water inhibit growth of *Euplotes vannus*, and the concentration become lethal from 7,870.5 mg L⁻¹. In fact, the capacity of these organisms to tolerate such toxic nitrogen compound is extraordinary, they are much more resistant than other live food organisms. However, *Euplotes* sp. fed only on baker's yeast could not possibly be offered alone to marine fish larvae due to its low nutritional value; it should be enriched mainly with polyunsaturated fatty acids before being offered as live food. Kraul (2006 apud CÔRTEZ; TSUZUKI, 2012) analyzed the composition of ciliates of the genus *Euplotes* fed enriched commercial diets and reported that they are capable of incorporating moderate levels of fatty acids (especially n-3 EPA and DHA). Nevertheless, such nutrient incorporation was lower than in rotifers and brine shrimp.

The density of *Euplotes* sp. fed Culture Selco was satisfactory although it is a product originally designed to feed rotifers. Even with a growth performance lower than the group fed baker's yeast, Culture Selco in the culture of *Euplotes* sp. may be interesting when the intention is to feed these organisms to fish larvae, as the product is enriched with polyunsaturated fatty acids.

Like other culture organisms, culturing *Euplotes* sp. has some advantages and disadvantages. Some of the advantages are the small size, fast growth, high culture densities, and resistance to high concentrations of ammonia in the water. The possible disadvantages may be that these protozoa need to be enriched before supplied to fish larvae, and their benthic habit may hinder predation (CHENG et al., 2004; XU et al., 2004). Additionally, the size of young and adult protozoa does not differ, so feeding other live food of larger size, such as rotifers, may be necessary as fish larvae grow.

Conclusion

The present study demonstrates the feasibility of an intensive monoculture of the ciliate protozoan *Euplotes* sp. Feeding microalgae *N. oculata* at a density

of $2.2 \pm 1.7 \times 10^6$ cells mL⁻¹ to these organisms was not efficient. Enhanced biomass growth may be obtained by feeding baker's yeast or Culture Selco 3000, products that are commonly used in rotifer culture.

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References

- CHENG, S. H.; AOKI, S.; MAEDA, M.; HINO, A. Competition between the rotifer *Brachionus rotundiformis* and the ciliate *Euplotes vannus* fed on two different algae. **Aquaculture**, v. 241, n. 26, p. 331-343, 2004.
- CÔRTEZ, G. F.; TUSUZUKI, M. Y. Efeito do tamanho do rotífero na sobrevivência e no crescimento de neon gobi *Elacatinus figaro* durante as fases iniciais de larvicultura. **Boletim do Instituto de Pesca**, v. 36, n. 3, p. 205-212, 2010.
- CÔRTEZ, G. F. ; TSUZUKI, M. Y. Effect of different live food on survival and growth of first feeding barber goby, *Elacatinus figaro* (Sazima, Moura & Rosa 1997) larvae. **Aquaculture Research**, v. 43, issue 6, p. 831-834, 2012.
- FIGUEIREDO, G. M.; NASH, R. D. M.; MONTAGNES, D. J. S. Do protozoa contribute significantly to the diet of larval fish in the Irish Sea? **Journal of the Marine Biological Association of the United Kingdom**, v. 87, n. 4, p. 843-850, 2007.
- HAGIWARA, A.; GALLARDO, W. G.; ASSAVAAREE, M.; KOTANI, T.; ARAUJO, A. B. Live food production in Japan: recent progress and future aspects. **Aquaculture**, v. 200, n. 1-2, p. 111-127, 2001.
- HOLT, J.; HOLT, S. A. Vertical distribution and role of physical processes in the feeding dynamics of two larval sciaenids *S. ocellatus* and *C. nebulosus*. **Marine Ecology Progress Series**, v. 193, p. 181-190, 2000.
- HUNT VON HERBING, I.; GALLAGER, S. M. Foraging behaviour in early Atlantic cod larvae (*Gadus morhua*) feeding on protozoan (*Balanion* sp.) and a copepod nauplius (*Pseudodiaptomus* sp.). **Marine Biology**, v. 136, n. 3, p. 591-602, 2000.
- HUNT VON HERBING, I.; GALLAGER, S. M.; HALTEMAN, W. Metabolic costs of pursuit and attack in early larval Atlantic cod. **Marine Ecology Progress Series**, v. 216, p. 201-212, 2001.
- KAMIYAMA, T. The impact of grazing by microzooplankton in northern Hiroshima Bay, the Seto Inland Sea, Japan. **Marine Biology**, v. 119, n. 1, p. 77-88, 1994.
- OLIVOTTO, I.; ZENOBI, A.; ROLLO, A.; MIGLIARINI, B.; AVELLA, M.; CARNEVALI, O. Breeding, rearing and

feeding studies in the cleaner goby *Gobiosoma evelynae*. **Aquaculture**, v. 250, n. 1-2, p. 175-182, 2005.

STATISTICA. **StatSoft, Inc.** Version 7.0. Tulsa, OK, United States of America, 2009.

STØTTRUP, J. G.; MCEVOY, L. A. **Live Feeds in Marine Aquaculture**. Oxford: Blackwell Science Ltd., 2003.

THOMPSON, F. L.; ABREU, P. C.; CAVALLI, R. The use of microorganisms as food source for *Penaeus paulensis* larvae. **Aquaculture**, v. 174, n. 1-2, p. 139-153, 1999.

WILKS, S. A.; SLEIGH, M. A. Grazing rates in *Euplotes mutabilis*: relationship between particle size and

concentrations. **Microbiology Ecology**, v. 36, n. 2, p. 165-174, 1998.

XU, H.; SONG, W.; WARREN, A. An investigation of the tolerance to ammonia of the marine ciliate *Euplotes vannus* (Protozoa, Ciliophora). **Hydrobiologia**, v. 519, n. 1-3, p. 189-195, 2004.

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