ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(3): 124-127

www.fisheriesjournal.com
Received: 06-03-2016
Accepted: 07-04-2016

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Effective method to culture infusoria, a highly potential starter feed for marine finfish larvae

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Abstract
This study was conducted to detect suitable protozoan species of infusoria as starter diet for early stage of marine finfish larvae. Infusoria were cultured using vegetables (*Brassica pekinensis* and *Brassica chinensis)* and dry fish meal with 30 ppt, 15 ppt saline water and freshwater in 40 liter aquaria. In the 30 and 15 ppt aquaria, *Euplotes* sp. was the dominant species and cell sizes were 60–80 µm. The densities of *Euplotes* sp. were 400–500 individuals/ml in the 30 ppt aquaria and 800-1300 individuals/ml in the 15 ppt aquaria. In both conditions, the high densities were maintained for 13 days. The densities of protozoa in saline water were higher in 15 ppt than in 30 ppt aquaria. The densities of protozoa cultured by our method was enough amount for early stage larvae of groupers and snappers. Therefore, this method is suitable for protozoa culture in small scale hatcheries.

Keywords: Fish larvae, Live feed, Infusoria, Protozoa, Starter diet

1. Introduction
Infusoria play an important role in microbial food chains [1]. Infusoria are the microscopic single celled animalcules belonging to Class, Ciliata and Phylum, Protozoa [2]. They are small in size, soft bodied and highly nutritious making them an ideal starter diet for early stage fish larvae [2]. In both freshwater and marine aquaculture, protozoa often constitute the sole food source during the earliest developmental stages [1].

Generally, to culture freshwater infusoria, an aquarium is filled with freshwater. Lettuce, cabbage, and banana peels are put into the aquarium to stimulate infusoria growth. After 4–5 days infusoria can be seen under a microscope and these have been used as starter foods for freshwater ornamental fish [2].

Infusoria culture methods in seawater were reported by Kitani [3]. In this method, minced radish leaves covered the entire water surface of 3 liter glass basins. Dry fish meal was placed on the leaves surface and aeration was not supplied. After approximately 10 days infusoria were observed. Rodes and Phelps [4] described the ciliate, *Fabrea salina* Hennenguy, 1890 culture method. They used filtered seawater in Erlenmeyer flasks to culture *F. salina* using the algae *Isochrysis* and *Rhodomonas* with no aeration. *F. salina* increased in the flask 3–4 days later.

In tropical regions, many important grouper and snapper species are used in aquaculture. These species larvae have small body sizes and small mouths. Rhodes and Phelps [4] studied larval rearing of red snapper *Lutjanus campechanus* (Poey, 1860) using nauplii including infusoria, and they got successful to rear the larvae. Nagano *et al* [5] also studied larval rearing of the convict grouper *Epinephelus septemfasciatus* (Thunberg, 1793) using ciliated protozoa. They concluded that the protozoa were an important food source to bridge the gap until fish larvae were ready to eat copepod nauplii.

Although the number of studies regarding infusoria are very limited compared with rotifers and other zooplankton, most studies mention the importance of infusoria or protozoa as starter diet for fish larvae for example Sanders and Wickham[6] and so on [7-9]. In the previous infusoria culture methods, Kitani's [3] method is unique and simple; however, the study did not provide detailed results. The present study was conducted to culture infusoria following Kitani's [3] method using different salinities of 0, 15, and 30 ppt. Protozoa densities were compared and protozoan genera were identified.

2. Materials and Methods

Infusoria were cultured following Kitani's [3] method. The present study was conducted in the laboratory at the Kulliyyah of Science, International Islamic University Malaysia. Seawater was brought from the hatchery at the Institute of Oceanography and Maritime Studies (INOCEM), International Islamic University, Malaysia.

Small dried anchovies (TL 40–60 mm) were purchased from a general dry fish market. Fish meal was made from the anchovy using a general food processor. Vegetables (*Brassica rapa* subsp. *pekinensis* (Lour.) Rupr. and *Brassica rapa* subsp. *chinensis* L.) were also purchased from a general market and cut into small pieces (1–2 cm). 10 g of fish meal (0.025%/water volume) and 200 g of vegetables (0.5%/water volume) were put into 0, 15, and 30 ppt fresh and saline water in 40 liter aquaria. Vegetables and fish meal were given to aquaria only one time at beginning, after that they were not added to the aquaria. Each salinity aquaria was in triplicate for a total of nine aquaria. Small vegetable pieces covered the entire water surface and fish meal was placed on the surface of the vegetables. Before we started culturing the infusoria, seawater from INOCEM was checked, and small number of protozoa were observed in the seawater. Water temperature was 26 °C–28 °C and dissolved oxygen was 0.2–0.3 ppm during infusoria culture.

Infusoria were observed to determine the genera and the densities of the dominant genus every 2 days for 13 days. Counting protozoa was done using a Sedgwick rafter cell under a light microscope. The identification of protozoan genus was determined by morphological characteristics such as body shape, presence/absence of appendages and setae or spines under a light microscope. Protozoan morphometric were determined for at least 20 cells.

3. Results

3.1. Protozoan genera

Protozoa were observed in all aquaria. The 15 and 30 ppt aquaria had the same dominant species (*Euplotes* sp.) and cell sizes were 60–80 µm (Figure 1a, b). The morphological characteristic of *Euplotes* sp. were the distinctive cirri on the cell surface. *Paramecium* sp. were dominant in the 0 ppt aquaria and cell size was approximately 100 µm (Figure 1c). When these freshwater protozoa were transferred to seawater (30 ppt), they shrank and became inactive.

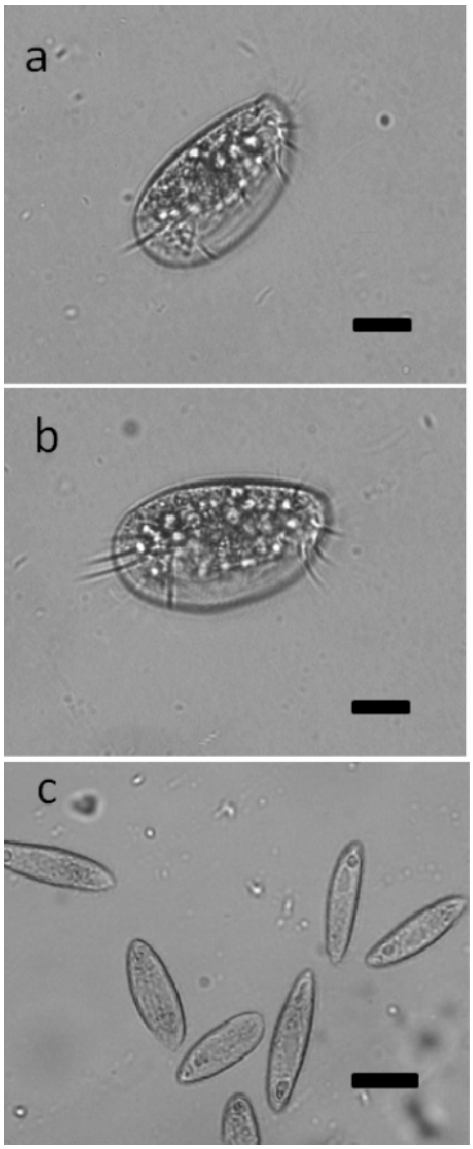

Fig 1: Photomicrographs of dominant infusoria species in different salinities (a) *Euplotes* sp. in 30 ppt, (b) *Euplotes* sp. in 15 ppt, (c) *Paramecium* sp. in 0 ppt. Scales are (a) 10 µm, (b) 10 µm, (c) 50 µm.

3.2. Protozoan density

Changes in protozoa densities are shown in Figure 2. The protozoa densities increased on day 3 following cultivation. The *Euplotes* sp. densities in the 30 ppt aquaria were 400–500 individuals/ml and the densities were maintained for 13 days. The *Euplotes* sp. densities in the 15 ppt aquaria were 800–1300 individuals/ml and these high level densities were maintained for 6–13 days. The *Paramecium* sp. densities in the 0 ppt aquaria were considerably high from day 1 following cultivation with peak densities ranging from 10000–15000 individuals/ml. However, the high densities decreased from day 7.

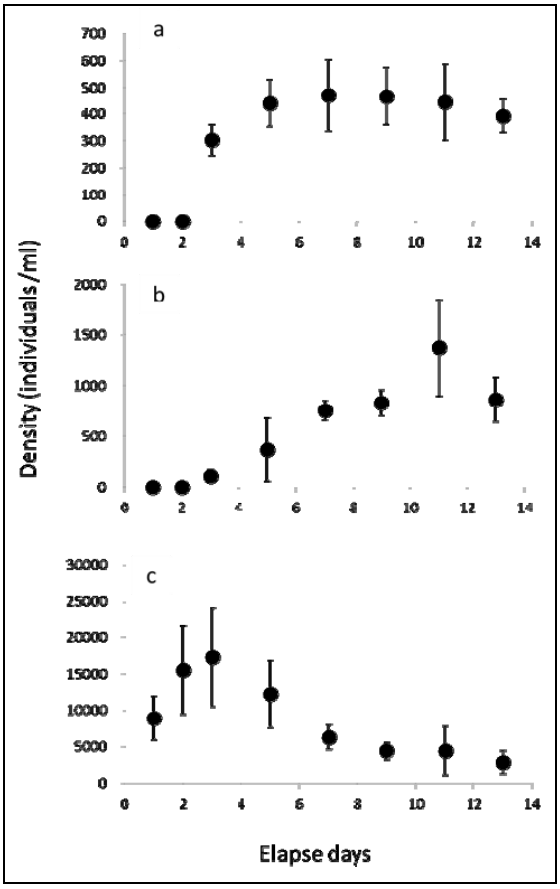

Fig 2: Densities (mean ± SD) of infusoria cultured in different salinities (a) 30 ppt, (b) 15 ppt, (c) 0 ppt

4. Discussion

In both 15 and 30 ppt aquaria, *Euplotes* sp. was the dominant. The densities of *Euplotes* sp. were higher in 15 ppt than in 30 ppt aquaria. High densities of *Euplotes* sp. continued for 1–2 weeks. Cortes *et al*. [10] cultivated *Euplotes* sp. with a salinity of 30 ± 2 ppt. Madhu and Madhu [11] also cultured *Euplotes* sp. with salinities ranging from 30–32 ppt. This suggests that *Euplotes* sp. can be cultured in saline water (15–30 ppt).

In freshwater aquaria, *Paramecium* sp. was dominant and the densities were very high that were 10000–15000 individuals/ml. However, if the *Paramecium* sp. was transferred to 30 ppt seawater, they shrank and became inactive under a microscope. Therefore they will be inactive in larval rearing tanks and cannot be used as live feed for marine finfish larvae.

In the present study, we used a common fish meal and vegetables. In other studies, Madhu and Madhu [11] cultured *Euplotes* sp. using micro algae, *Nannochloropsis oculata* (Droop) Hibberd, 1981 and *Isochrysis galbana* Parke, 1949. Pedrazzani *et al*. [12] also cultured *Euplotes* sp. using a paste of *N. oculata*. Cortes *et al*. [10] cultured *Euplotes* sp. using three different kinds of feeds, *N. oculata*, Selco (for rotifer culture), and baker's yeast. The *N. oculata* was monitored and kept at 2.2 ± 1.7 x 10^6 cells mL^{-1}. Baker's yeast and Selco were fed at a concentration of 0.5 g/million protozoa once a day. The salinity was 30 ± 2 ppt, aeration was constant, and there was no water renovation. The densities of *Euplotes* sp. using *N. oculata* was 2300 individuals/ml, using baker's yeast it was 15000 individuals/ml, and using Selco it was 11000 individuals/ml.

The method of Cortes *et al*. [10] produced much higher densities than our methods. However, our culture method is simpler to culture the *Euplotes* sp. than Cortes *et al*.'s [10] method. Our method produced densities of 500–1000 individuals/ml which is enough to feed early stage grouper and snapper larvae in small scale larval production. Our method needs only beginning operation. Later on, it does not require operation, for instance, no adding vegetables and fish meal. Moreover, it does not require high cost facilities or high level techniques to culture infusoria, so this is suitable method for live feed culture for small scale hatcheries.

In general, fishes in higher temperature region produce a larger number of smaller eggs. In tropical region marine finfish larvae, for example, groupers and snappers, have tiny mouths with an opening less than 100 µm. Therefore, we have to detect small size zooplankton. Although there are many fish species candidates for aquaculture in tropical regions, the small mouth sizes of early stage larvae make the seed production difficult and unstable. Protozoa of infusoria are useful live feed that solves this problem making it possible to culture many kinds of valuable marine finfish larvae. A simple and small facility is enough to culture protozoan of infusoria; therefore, protozoa are a useful live feed for the larval culture of marine finfish in tropical region.

Further study is needed to investigate larval rearing using the protozoa. However, in a preliminary study we were successful with seed production of the brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775). The early stage of the larvae grew by the protozoa (Mukai unpublished data). Therefore this method is suitable for live feed culture in small scale hatcheries.

5. Conclusion

A simple and effective culture technique is important to enhance fish production in aquaculture industry. This study method could produce useful protozoan, *Euplotes* sp. as starter feed for marine finfish larvae. This method is simple and effective, and furthermore, the method does not require a sophisticated facilities making it suitable for small scale hatcheries.

6. Acknowledgement

This study was supported by Fundamental Research Grant Scheme of Ministry of Education Malaysia.

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