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Effect of Short-Term Enrichment of Wild Zooplankton on Survival of Larval Maroon Clownfish (*Premnas biaculeatus*)

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Abstract: The use of a 3-hour enrichment of wild zooplankton as a food for larval maroon clownfish (*Premnas biaculeatus*) was investigated. Larval growth and survival were evaluated by assessing total length and mortality. The larvae were fed three different types of feed: wild plankton without enrichment, wild plankton enriched with cultured microalgae and wild plankton enriched with Protein Selco®. Growth was nearly identical among treatments, averaging (SD) 4.78 (0.0), 4.84 (0.1) and 4.79 (0.07), respectively. Survival rate was higher in larvae fed the algal-enriched plankton than in larvae fed the other two feeds. Mortality was highest (100%) in larvae fed un-enriched wild plankton; mortality was lower (70%) in larvae fed the Protein Selco®-enriched diet and lowest (40%) in larvae fed the microalgae-enriched diet.

Key words: Wild plankton · Microalgae · Protein-Selco® · Enrichment · Growth and survival

INTRODUCTION

Many ornamental marine invertebrates and fish are captured from Southeast Asia and other areas in the Indian and Pacific oceans and transported to customers in the United States, Europe and Japan often with negative consequences for their survival and the sustainable use of coral reefs [1]. One option for decreasing the wild harvest is captive breeding [2-5].

Clownfish or anemone fish are in the family Pomacentridae. They are the first marine fish to be bred successfully in captivity. Appropriate diet is an essential element of success in larval rearing [5-7]. Rotifers and *Artemia nauplii* are usually used as live food, but contain low concentrations of omega-3 fatty acids and carotenoids. To overcome this shortfall, rotifers and brine shrimp for use in feeding larval fishes are often enriched with lipid-containing algae and/or artificial enriching agents [5, 8- 13]. Few studies have been performed on the use of wild plankton as a food source for fish larvae [14-18]. Usually, the enrichment process takes 24 hours, which is not a problem when feeding plankton reared in the hatchery, but which is difficult when collecting and holding wild zooplankton on a daily basis. The goal of this study was to determine the effect of feeding rapidly-enriched (3 hours) wild zooplankton on the growth and survival of larval maroon clownfish.

MATERIALS AND METHODS

Spawning: Larvae from three spawning pairs of *P. biaculeatus* along with its host anemone, *Stichodactyla mertensii*, reared at the marine ornamental fish hatchery at the Centre of Advanced Study in Marine Biology, Annamalai University, were selected for this study.

Larval Rearing: Nine fiber glass reinforced plastic tanks (FRP, 50 L) containing 30 L of filtered estuarine water were stocked with 100±10 two-hour old larvae. For the first 2 days post hatch (DPH), the larvae in all tanks were fed only *Nannochloropsis salina* enriched wild zooplankton, then the tanks were divided into three groups each of three tanks. The first group (Treatment 1) were fed wild plankton (20 individual/ml) without any enrichment, the second group (Treatment 2) were fed Protein-Selco®-enriched wild zooplankton and the third

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group (Treatment 3) were fed wild zooplankton enriched with algae (*N. salina*). Ten percent of the water was exchanged from each tank before feeding in the morning to maintain water quality. Water temperature was maintained at 28–30°C, salinity was maintained at 22–24 ppt, pH at 7.5–7.8, dissolved oxygen at 5.0–5.5 mg/l (through mild aeration) and photoperiod was 12 hour light: 12 hour dark with a light intensity of 2,500–3,000 lux maintained with a 5 watt fluorescent bulb attached to each tank 20 cm above water level.

Collection of Wild Plankton: Wild zooplankton were collected daily during morning and evening hours from the Vellar Estuary (11°29′ N, 79°46′ E) along the southeastern coast of Tamil Nadu using a plankton collection net (50 µm) towed behind a motorized boat. The collected zooplankton was washed thoroughly with treated estuarine water 2–3 times before feeding to fish.

Enrichment: For Treatment 2, Protein Selco® was purchased from a chemical supplier (INVE Technologies, Belgium) and 0.20 mg was mixed well with 100 ml of water into which wild zooplankton were introduced at a density of 20 ind/ml and allowed to swim for 3 hours to induce enrichment. For Treatment 3, filtered wild zooplankton at a density of 20 ind/ml were enriched for three hours in a solution of 100 ml of the microalgae *N. salina* ($1.0x10^6$ cells/ml) and kept for 3 hours with mild aeration.

The study was carried out over 15 days, at which point surviving larvae were counted and measured for total length using an ocular micrometer (Erma, Tokyo, Japan) under a microscope (Novex, Holland). Treatments were compared using one-way ANOVA.

RESULTS

Growth: After 15 day, lengths of the larvae fed un-enriched wild zooplankton ranged from 3.7 to 4.0 mm with a mean (SD) of 3.78 (0.0). Lengths of the larvae fed algal-enriched zooplankton ranged from 3.7 to 4.06 mm with a mean (SD) of 3.84 (0.1) and lengths of the larvae fed Protein Selco®-enriched zooplankton varied from 3.7 to 4.1 mm with a mean (SD) of 3.79 (0.07) (Fig. 1). There were no significant differences among treatments.

Survival: In the present study, among the three live feeds provided, survival rate was zero in the larvae fed enriched wild plankton, 30% in the larvae fed Protein Selco®-enriched plankton (in which 3 d duration enriching) and 60% in the larvae fed algal-enriched plankton (in which 3 d duration enriching). The one-way ANOVA showed a significant difference between the wild, Protein Selco®-enriched and algal-enriched diets ($P<0.05$). The mortality in larvae fed un-enriched wild plankton was 1.0 (1.0); maximum mortality was observed on the sixth day and no mortality occurred on days 4, 5, 8 and 10. The mortality in larvae fed Protein Selco®-enriched plankton was 0.7 (0.8); higher mortality was observed on the third and ninth days and no mortality occurred on days 4, 5, 7, 8, 11 and 12. The mortality in larvae fed algal-enriched plankton was 0.4 (0.7); highest mortality was observed on the sixth day and no mortality occurred on days 7, 8, 10, 11 and 12. In the current study, the larval survival rate in the three different experimental tanks was 40.71%, 52.80% and 85.14% for un-enriched, Protein Selco®-enriched and algal-enriched plankton, respectively. Larvae in experiment 3 accepted *Artemia nauplii* at day 14 post hatching, whereas larvae in the other experiments accepted the nauplii at day 16 post hatching.

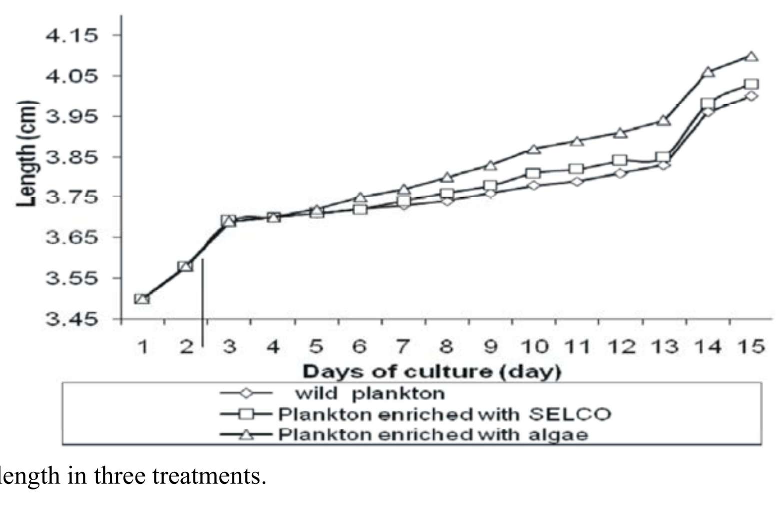

Fig. 1: Larval total length in three treatments.

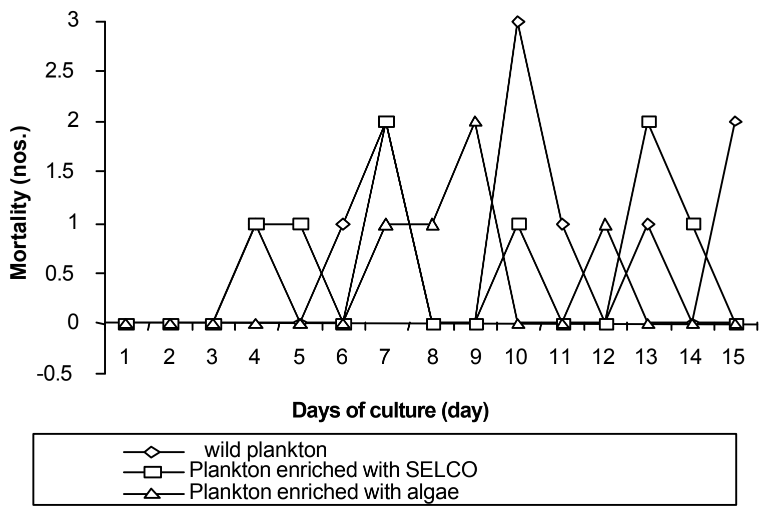

Fig. 2: Larval mortality in three enrichments

DISCUSSION

Enriching rotifers with DHA is effective for the growth and survival of early larvae of marine fish [9]. If marine fish larvae are fed poorly nourished rotifers as a first food (e.g., algae only), however, larvae do not survive [4]. Providing the proper concentrations of PUFAs (e.g., DHA) in the diets of early larval stages increasingly improves nervous system function [4, 5, 19]. Milk fish larvae given DHA-enriched (with DHA Protein Selco) live food showed higher survival rates than those given a non enriched diet; larval deformity was diminished as well [20]. When Senegal sole larvae were fed DHA Protein Selco®-enriched *Artemia*, they grew significantly longer than larvae given ARA-enriched Artemia, but no significant difference in survival rate was observed [21]. Gapasin and Duray [19] reported that DHA, concentrations in rotifers enriched with DHA Protein Selco were higher than DHA concentrations in rotifers cultured with algal feed (*Chlorella*). Some experiments conducted by Avella *et al*. [9] demonstrated higher growth in false percula clownfish larvae when they were fed enriched (with AlgaMac-2000) *Artemia nauplii*.

Although in this work the wild plankton have only enriched for 3 hours that seem to be short time than common procedure (24 hour), our finding demonstrated that in case the feed let to be enriched they will initially tend to feed more fresh algae than artificial ones. As survival and growth of larval fish as long as fed algal-enrichment were considerably higher than the others.

Our data also showed that the highest survival rate of maroon clownfish larvae fed an algal-enriched wild plankton was 85.14, whereas the highest survival rate of Clarkii clownfish fed a combination of rotifers, copepod nauplii, *Artemia nauplii* and copepodites–copepods was 55 [22]. The survival rate of sea bream larvae also was highest when fed a combination of algae mixture, Super Selco and DHA Protein Selco [23]. No significant difference was observed when sunshine bass larvae were fed enriched rotifers (with *Nannocloropsis* plus Culture Selco 3000 compared to *Nannocloropsis* only); however, larvae showed more growth when fed *Nannocloropsis sp*. plus Culture Selco 3000 compared to *Nannocloropsis* only [13].

In conclusion we can state a variety of live food as wild plankton would definitely improve the growth and survival of fish larvae.

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