PHILIPP AGRIC SCIENTIST
Vol. 104 No. 4, 388-395
December 2021

ISSN 0031-7454

Rearing Silver Therapon *Leiopotherapon plumbeus* (Teleostei: Terapontidae) Larvae Using Euryhaline Rotifers as Starter Food

Frolan A. Aya[1,*], Vicar Stella N. Nillasca[1], and Luis Maria B. Garcia[2]

[1]Binangonan Freshwater Station, Aquaculture Department, Southeast Asian Fisheries Development Center, Binangonan, Rizal 1940, Philippines
[2]Institute of Biology, College of Science, University of the Philippines Diliman, Quezon City 1101, Philippines

[*]Author for correspondence; Email: faya@seafdec.org.ph; Tel.: (63-2) 219-2696

Received: January 20, 2021/ Revised: October 11, 2021/ Accepted: November 29, 2021

The silver therapon *Leiopotherapon plumbeus* is an important but dwindling freshwater food commodity in the Philippine freshwater habitats. The influence of feeding regimes on growth performance and survival of first-feeding silver therapon larvae fed euryhaline rotifers (*Brachionus rotundiformis* and *B. plicatilis*) as starter food was examined. Larvae at 2 days post-hatch (DPH) (1.93 ± 0.07 mm; 200 larvae/basin) were initially reared on rotifers for 12 days followed by *Artemia* nauplii from 14 to 35 DPH as follows: (A) *B. rotundiformis* from 2-13 DPH; (B) *B. rotundiformis* from 2-7 DPH and *Moina micrura* from 8-13 DPH; and (C) *B. plicatilis* from 2-13 DPH. After 35 days of rearing, mean survival rates were significantly higher in larvae fed *B. rotundiformis* (69.2%) than those co-fed *B. rotundiformis* and *M. micrura* (34.6%) or *B. plicatilis* alone (26.3%). Higher ingestion rates were observed for *B. rotundiformis*-fed larvae (1.6 ± 0.5 to 4.4 ± 0.5 ind larvae^{-1}) than larvae fed *B. plicatilis* (0.0 to 3.2 ± 0.8 ind larvae^{-1}) during the critical initial feeding stage. However, larvae fed *B. plicatilis* (20.75 ± 0.48 mm) were significantly longer than those fed *B. rotundiformis* alone (15.62 ± 0.40 mm) or co-fed *B. rotundiformis* and *M. micrura* (18.57 ± 0.58 mm). The fastest growth was observed in larvae fed *B. plicatilis*, with length increment (LI) and specific growth rate (SGR) of 18.8 mm and 6.8% day^{-1}, respectively. Eye diameter, head length, snout length and pre-anal length increased but were not affected when larvae were fed two rotifer species. These results demonstrate that feeding euryhaline rotifer *B. rotundiformis* from 2 to 13 DPH followed by *Artemia* is a suitable feeding regime for better survival of silver therapon larvae under laboratory rearing conditions.

Keywords: *Leiopotherapon plumbeus*, first feeding, growth, live feeds, larvae

Abbreviations: DPH – days post-hatch, TL – total length, LI – length increment, SGRL – length-based specific growth rate, SEAFDEC/AQD – Southeast Asian Fisheries Development Center, Aquaculture Department

INTRODUCTION

The silver therapon *Leiopotherapon plumbeus* (Kner 1864) is an important freshwater food fish species found in Laguna de Bay, Philippines, comprising about 70% of the total fishery catch (Mercene and Cabrera 1991). However, the wild fishery catch of this species has declined significantly as a result of overfishing and habitat degradation (Palma et al. 2002), and introduction of invasive alien species (Guerrero 2014). Recently, silver therapon is increasingly considered an emerging species for domestication (Garcia et al. 2020), and culture of this species may help conserve fish biodiversity and possibly provide an alternative source of food and livelihood in rural areas (Aya et al. 2016).

Previous studies on silver therapon focused mainly on hormone-induced spawning (Denusta et al. 2014), early life history (Aya et al. 2016, 2017), and some aspects of feeding, growth and mouth morphology (Aya et al. 2015; Aya and Garcia 2016; Aya et al. 2019, 2021). However, the lack of a suitable feeding regime remains one of the bottlenecks in the intensive culture of silver therapon in the hatchery. During its early life history, newly hatched silver therapon larvae measure only 1.8 mm, have limited endogenous yolk reserves, and a small mouth size (Aya et al. 2016; Garcia et al. 2020), requiring small live food organisms to avoid irreversible starvation (Yanes-Roca et al. 2018; Garcia et al. 2020).

The start of exogenous feeding is a critical period in the development of a fish, accounting for the majority of

larval mortalities (Yúfera and Darias 2007). Indeed, previous larval rearing trials resulted in mass mortalities at 2 days post-hatch (DPH), the onset of exogenous feeding in silver therapon larvae (Aya et al. 2016). The survival of hatchery-reared fish larvae with small mouths will therefore depend on the provision of suitable live food prey (Shirota 1970; Cunha and Planas 1999; Yúfera and Darias 2007). Two euryhaline rotifer species, *Brachionus rotundiformis* (105-250 μm in lorica length; Haberman and Sudzuki 1998) and *B. plicatilis* (123-292 μm in lorica length; Snell and Carillo 1984), have been used as initial live food prey for rearing marine fish larvae due to their small size and slow motility (Lubzens et al. 1989; Okumura 1997; Hagiwara et al. 2001, 2007; Soyano et al. 2008). However, rotifers have deficiencies in long-chain highly unsaturated fatty acids (HUFAs) (Watanabe et al. 1983), and so enrichment of live food with nutritional supplements (Eryalçın 2018, 2019) is necessary before they are fed to the fish larvae. To date, the use of euryhaline rotifers is only limited to a few freshwater food fish species such as cyprinid larvae, where an increase in growth was observed when fed *B. plicatilis* (Lubzens et al. 1987). Rotifer species, *B. plicatilis* in particular, can tolerate a wide range of salinities (2-97 ppt) (Lubzens et al. 1995). As silver therapon also thrives in brackishwater, the possible use of these euryhaline rotifer species in long-term early feeding stages may improve the growth, development, and survival of the fish larvae. Preliminary results of a short feeding trial of 2-4 DPH silver therapon larvae on these rotifer species showed that first-feeding larvae survived, suggesting they were able to ingest both rotifer species, despite the larger size of *B. plicatilis*.

This study aims to examine the influence of rotifer-based feeding regimes on growth performance and survival of first-feeding silver therapon larvae to support the development of a hatchery rearing protocol for this important freshwater food fish.

MATERIALS AND METHODS

Production of Live Food

Green alga *Chlorella ellipsoidea* was mass-produced in 300 L polyethylene tanks. Algal starters were subsequently inoculated into 10 L plastic round basins filled with 5 L of non-chlorinated ground tap water, fertilized with ammonium phosphate (16-20-0) at 0.1 g L^{-1}, and provided with 24 h illumination and mild aeration until the maximum algal density (10^6 cells mL^{-1}) was reached.

Rotifers (*Brachionus rotundiformis* and *B. plicatilis*) were cultured separately without enrichment in several 500 mL to 1 L flasks using low salinity medium (2 ppt) and provided with *C. ellipsoidea* as food. After 5 days, rotifers were harvested, concentrated using 57 μm mesh-sized plankton net, and inoculated into the rearing plastic basins. Artificially prepared seawater (12 ppt) was then added to the rearing water at a final concentration of 4-5 ppt.

Moina micrura was produced in 20 L capacity glass aquaria without aeration and fed *C. ellipsoidea*. *M. micrura* was harvested after 5-8 days of culture, sieved with 80 μm plankton net, counted, and introduced into the rearing basins according to the experimental design.

Hatching of *Artemia* cysts was performed according to Lavens and Sorgeloos (1996), and unenriched newly-hatched nauplii were used for feeding the larvae.

Larvae and Feeding Experiments

Larvae were obtained from hormone-induced spawning of captive silver therapon broodstock (Aya et al. 2015, 2016) consisting of one female paired with two males maintained in outdoor concrete tanks at the hatchery facility of the Binangonan Freshwater Station of SEAFDEC Aquaculture Department.

Newly hatched larvae (1.93 ± 0.07 mm in total length (TL)) were stocked at 25 larvae per liter (or 200 larvae per basin) each in four replicate 10 L round plastic basins provided with mild aeration. Experimental basins were set up indoors provided with an overhead 32 watt fluorescent lamp to simulate natural photoperiod (around 12 h light and 12 h dark) conditions. The three feeding regimes were tested in quadruplicate (Fig. 1) as follows: (A) larvae were fed *B. rotundiformis* at 25 ind mL^{-1} from 2 days post-hatch (DPH) to 7 DPH and at 20 ind mL^{-1} from 8 to 13 DPH; (B) larvae were fed *B. rotundiformis* at 25 ind mL^{-1} from 2 to 7 DPH followed by *Moina micrura* at 5 ind mL^{-1} from 8 to 13 DPH; (C) larvae were fed *B. plicatilis* at 25 ind mL^{-1} from 2 to 7 DPH and at 20 ind mL^{-1} from 8 to 13 DPH. Thereafter, all treatment groups were fed *Artemia* nauplii from 14 to 35 DPH at various densities per period: 1 nauplii mL^{-1} from 14-20 DPH, 3 nauplii mL^{-1} at 21-27 DPH, and 5 nauplii mL^{-1} from 28-35 DPH. During the *Brachionus*-feeding days, *C. ellipsoidea* was added daily at 0.3 × 10^6 cells mL^{-1} as food and as a water conditioner. Feeding was performed three times daily at 9:00 a.m., 1:00 p.m., and 4:00 p.m. Feeding trial lasted for 35 days.

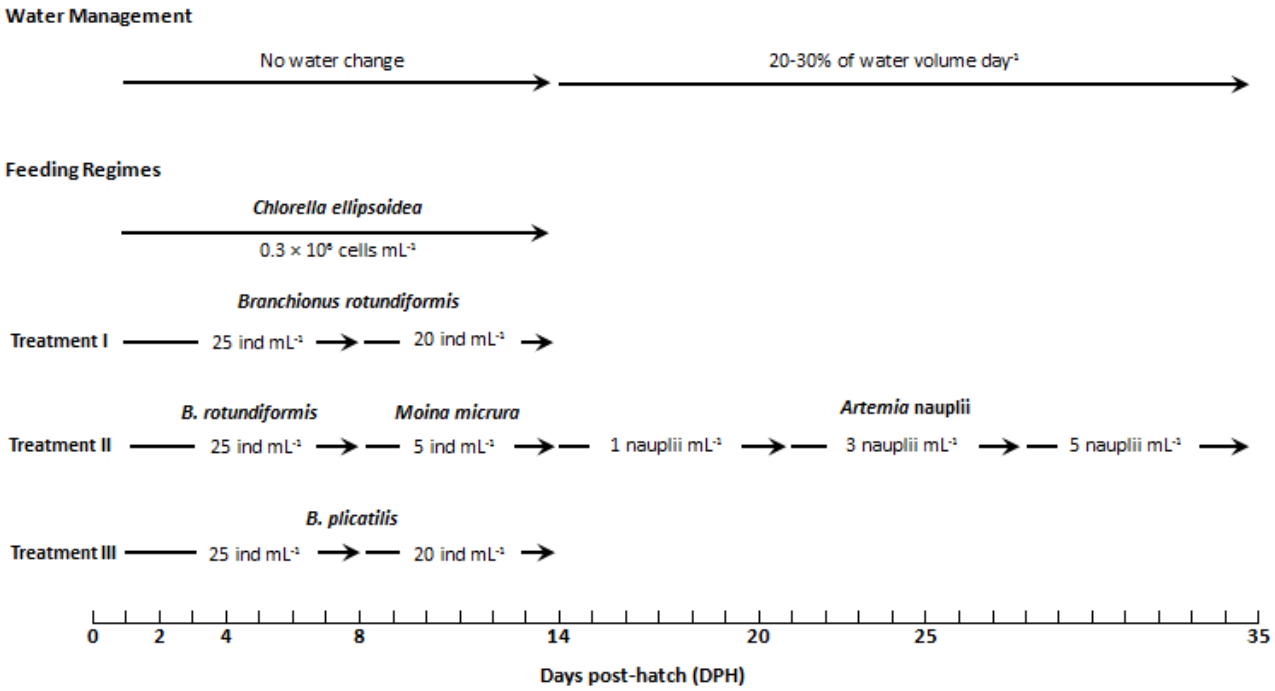

Fig. 1. Water management and feeding regimes in rearing first-feeding silver therapon *Leiopotherapon plumbeus* larvae.

Ingestion Rates and Morphological Development of First-Feeding Larvae

To examine the ingestion rates and morphological characters (total length (TL), body depth (BD), head length (HL), eye diameter (ED), snout length (SnL), and pre-anal length (PAL)) of silver therapon larvae from 2 to 13 DPH, larvae (200 larvae per basin) were stocked in additional four plastic basins (duplicate basins per rotifer species) and fed *B. rotundiformis* or *B. plicatilis* of similar concentration as described above.

Water Quality Management

During the rotifer feeding days, the rearing water was not changed (Fig. 1) although dead larvae and other debris found at the basin bottom were removed using a glass pipette before the morning feeding period. From 14 to 35 DPH, during which feeding shifted to *Artemia*, daily water renewal was 20-30% water volume per day. Water temperature and dissolved oxygen were measured every 2 days with a YSI dissolved oxygen meter (Yellow Springs, Ohio, USA) while *pH* and Total Ammonia Nitrogen were determined weekly using a *pH* meter (Beckman Model Phi 50) and an Aquarium Pharmaceuticals API Water Test Kit (MARS Fishcare, USA). Water temperature ranged from 25.2-30.5°C, dissolved oxygen between 5.34 and 7.84 mg L^{-1}, *pH* at 8.4-9.1, and Total Ammonia Nitrogen (TAN) from 0 to 0.5 mg L^{-1}.

Fish Sampling and Analysis of Samples

Ten larvae from each experimental basin were sampled at 6, 13, 18, 24, and 35 DPH, their length measured with a calibrated ocular micrometer (nearest 0.01 mm) or a digital caliper (± 0.01 mm), and then immediately returned to the same experimental basins. Survival was recorded by counting the remaining larvae in the experimental basins at 13 and 35 DPH.

To determine the feeding incidence (percent of larvae with rotifers in the gut) and the number of *Brachionus* ingested from first-feeding to 13 DPH larvae, samples of five larvae from each replicate basin of *B. rotundiformis* and *B. plicatilis*-fed groups were collected daily from 2 to 13 DPH and immediately preserved in 5% buffered formalin and the gut dissected. The same preserved larval samples were used for measurements of morphometric characters (in mm): TL, BD, HL, ED, SnL, and PAL. These morphometric characters were measured under a dissecting microscope with a calibrated ocular micrometer (to the nearest 0.01 mm).

Data and Statistical Analysis

Total length increment (LI (mm) = final total length – initial total length), specific growth rate of length (SGRL = 100 × (ln final length – ln initial length)/ days)), and survival (actual count at harvest / initial stock × 100) were determined. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test when

significant differences were detected. Percentage data were arcsine-transformed prior to statistical analysis. All tests were done at a level of significance of $P < 0.05$.

RESULTS AND DISCUSSION

First-feeding silver therapon larvae fed *B. rotundiformis* from 2 to 13 DPH had significantly better survival (73.9%) than those co-fed *B. rotundiformis* and *M. micrura* (34.6%) or *B. plicatilis* alone (44.8%) (Fig. 2). At 35 DPH, larvae fed *B. rotundiformis* still showed significantly higher survival (69.2%) than those fed *B. plicatilis* alone (26.3%), while larvae co-fed on *B. rotundiformis* and *M. micrura* had intermediate survival rates (34.2%) (Fig. 2; Table 1). The highest survival from larvae fed euryhaline rotifer *B. rotundiformis* demonstrates the suitability of this rotifer species as starter food for first-feeding silver therapon larvae. Similar results were obtained for early stage tropical marine fish larvae, such as grouper *Epinephelus suillus* (Duray et al. 1997). Meanwhile, feeding on a combination of *B. rotundiformis* and *M. micrura* resulted in an intermediate survival, suggesting that silver therapon larvae may require a relatively lengthy period of feeding on small-sized rotifers to improve survival.

Feeding incidence of silver therapon larvae on *B. rotundiformis* already reached 100% at 2 DPH, while those given *B. plicatilis* did not reach 100% until 3 DPH. From 2 to 4 DPH, larvae could ingest *B. rotundiformis* (1.6 ± 0.5 to 4.4 ± 0.5 ind larvae^{-1}) higher than *B. plicatilis* (0.0 to 3.2 ± 0.8 ind larvae^{-1}), but the difference between groups was not significant (Fig. 3). Ingestion of both species intensified from 5 to 9 DPH with a 7.5 and 2.7 fold increase for *B. rotundiformis* and *B. plicatilis*, respectively. It has been suggested that the food value of rotifers as prey for fish larvae depends on their size and nutritional composition (Tanaka et al. 2005; Akazawa et al. 2008), which have direct effects on feeding success, larval growth and survival (Yanes-Roca et al. 2018). The high survival of the first-feeding larvae on *B. rotundiformis* from this study was therefore attributed, in part, to the higher counts of *B. rotundiformis* than *B. plicatilis* in the gut of 2 to 9 DPH larvae, which was easily captured by the larvae due to the rotifer's smaller body size (116 μm in length and 98 μm in width). However, poor survival obtained in larvae fed *B. plicatilis* may have been due to the rotifer's slightly larger body size (130 μm in length and 114 μm in width) than *B. rotundiformis* which resulted in lower ingestion rates by first-feeding larvae. From this result, it can be argued that only larvae of silver therapon with bigger mouth gape size were able to capture and ingest *B. plicatilis*, indicating that prey dimension and foraging ability are limiting larval survival for this treatment. Likewise, low survival of pikeperch *Sander lucioperca* larvae fed a mixed diet of *B. plicatilis* and *Artemia nauplii* was reported due to prey size effect during the first 4-5 days of feeding (Yanes-Roca et al. 2018). However, in silver therapon larvae, ingestion on *B. rotundiformis* decreased to 11.8 ± 7.4 ind larvae^{-1} whereas active feeding on *B. plicatilis* (17.2 ± 20.3 to 25.3 ± 22.0 ind larvae^{-1}) by the larvae remained at 13 DPH (Fig. 3). Higher ingestion of larvae on *B. plicatilis* compared with that of *B. rotundiformis* after 9 DPH could thus be due to improved feeding efficiency as a result of increased mouth gape size of the larvae. At this point, the introduction of bigger prey such as *B. plicatilis* is necessary to sustain survival and growth of larvae (Zambonino Infante and Cahu 2007; Yanes-Roca et al. 2018).

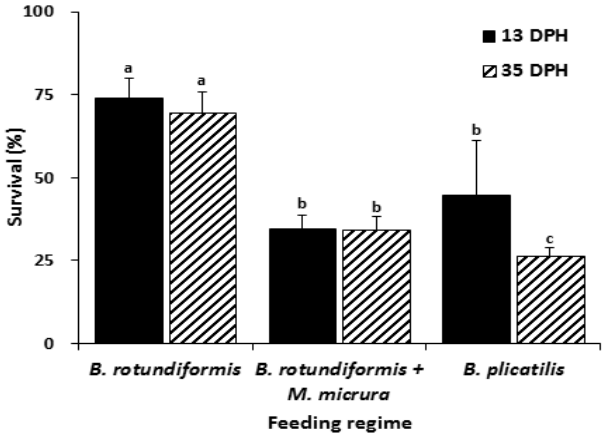

Fig. 2. Survival of first-feeding silver therapon *Leiopotherapon plumbeus* larvae fed *B. rotundiformis*, *B. rotundiformis*, and *M. micrura*, or *B. plicatilis* from 2 to 13 days post-hatch (DPH), and *Artemia* nauplii from 14 to 35 DPH. Means (± standard deviations, n = 4) with a different superscript are significantly different ($P < 0.05$).

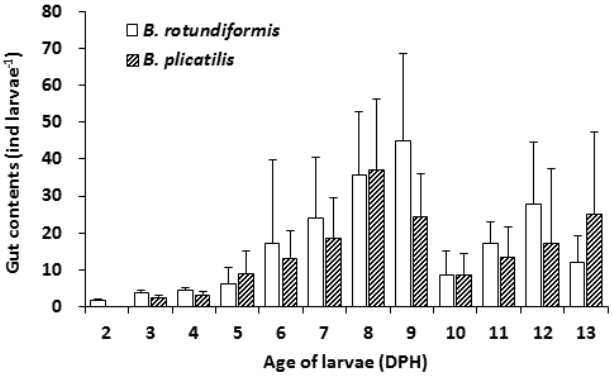

Fig. 3. Ingestion rates of first-feeding silver therapon *Leiopotherapon plumbeus* larvae fed *B. rotundiformis* or *B. plicatilis* from 2 to 13 days post-hatch (DPH). Vertical lines above bars represent standard deviations of the means, n = 10.

Table 1. Larval performance of silver therapon *Leiopotherapon plumbeus* under different feeding regimes after 35 days of rearing.

Parameters	Feeding Regime[1]		
	B. rotundiformis	*B. rotundiformis* + *M. micrura*	*B. plicatilis*
Survival (%)	69.20 ± 6.50[a]	34.62 ± 4.07[b]	26.30 ± 2.40[c]
TL (mm)	15.62 ± 0.40[c]	18.57 ± 0.58[b]	20.75 ± 0.48[a]
LI (mm)	13.69 ± 0.40[c]	16.40 ± 0.58[b]	18.82 ± 0.48[a]
SGRL (% day^{-1})	5.97 ± 0.06[c]	6.47 ± 0.09[b]	6.79 ± 0.07[a]

TL, total length; LI, length increment; SGRL, length-specific growth rate.

[1]Row means with a different superscript are significantly different ($P < 0.05$). Data are mean ± standard deviation; n = 4. Silver therapon *Leiopotherapon plumbeus* larvae at stocking had an initial TL of 1.93 ± 0.07 mm.

Although silver therapon larvae ingested a higher number of *B. rotundiformis* than *B. plicatilis* during the critical initial feeding stage, larval growth was comparable among the three treatment groups during the initial 12 days prior to *Artemia* feeding (Fig. 4). However, after transitioning to *Artemia* feeding from 14 to 35 DPH, larvae fed *B. plicatilis* sustained positive growth (Fig. 4; Table 1), which was significantly higher than the other two treatment groups. In addition, the total LI and SGRL differed among first-feeding regimes and were highest in larvae fed *B. plicatilis* (Table 1). Clearly, variations in body size and biomass of two rotifer species are two of the factors that determined the marked growth rate difference between treatment groups (Wullur et al. 2011; Yanes-Roca et al. 2018). Capturing prey is an energy-demanding process, and the growing fish larvae now required fewer but larger prey to obtain

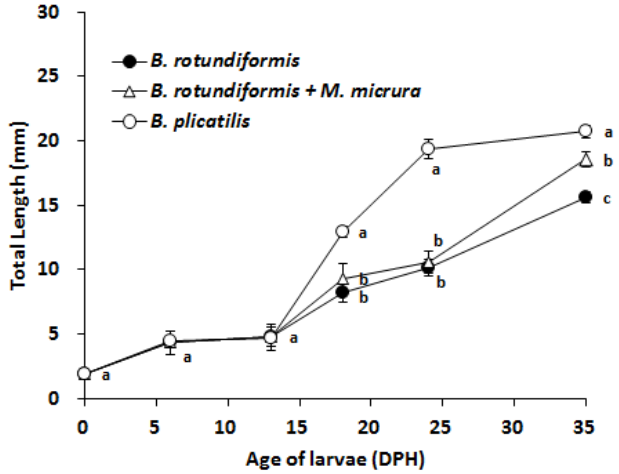

Fig. 4. Growth in terms of total length of first-feeding silver therapon *Leiopotherapon plumbeus* larvae fed *B. rotundiformis*, *B. rotundiformis*, and *M. micrura*, or *B. plicatilis* from 2 to 13 days post-hatch (DPH), and *Artemia* nauplii from 14 to 35 DPH. Means (± standard deviations, n = 4) with a different superscript are significantly different ($P < 0.05$).

an equal amount of energy (Duray et al. 1997), thus, explaining in part the higher growth rate of the larvae fed *B. plicatilis*. In addition, competition for available prey (Lundberg and Persson 1993) is likely one of the factors that could influence larval growth from this study. Regardless of larval density after the rotifer feeding days, the three treatment groups received equal levels of *Artemia* nauplii per volume of rearing water to give the remaining survivors an equal chance to capture their prey (Yanes-Roca et al. 2018). In this case, since the larvae fed *B. plicatilis* alone or *B. rotundiformis* and *M. micrura*, had relatively lower number of survivors, larvae in these two treatments were therefore exposed to sufficient density of *Artemia* nauplii resulting in optimal growth. However, the significantly lower growth in larvae fed *B. rotundiformis* alone was due to the higher number of surviving larvae in this treatment group competing for the available live food prey.

The nutritional value of live food organisms is also critical to the growth and survival of first-feeding larvae (Park et al. 2006), particularly the essential fatty acids which are rapidly diminished once their endogenous yolk energy reserves are completely exhausted to initiate exogenous feeding (Evans et al. 2000). In the present study, the fatty acid composition of live food organisms was not examined which would have explained in part the observed differences in larval survival between treatment groups. In the study of Seiffert et al. (2001), the levels of highly unsaturated fatty acids (HUFA) such as eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) observed in *B. rotundiformis* were higher than the levels found by Villegas (1990) in *B. plicatilis*. Similarly, Cabrera et al. (2005) found these highly unsaturated fatty acids at higher levels among small-sized rotifer. The differences in survival rates may be related to the differences in the dietary fatty acid composition of the rotifer species. *B. rotundiformis* may have provided the silver therapon larvae with nutrients essential for sustaining higher survival up to 13 DPH (Yanes-Roca et al. 2018), which *B. plicatilis* could not provide. Meanwhile, the type of rotifer species however did not influence the development of some morphometric characters such as eye diameter, head length, snout length, and pre-anal length during early feeding stages (Fig. 5).

This study adds critical information to the growing body of information on the feeding requirements of first-feeding silver therapon larvae useful in the development of reliable hatchery techniques for this species. The results suggest the suitability of the euryhaline rotifer *B. rotundiformis* as a starter food for first-feeding up to 13 DPH

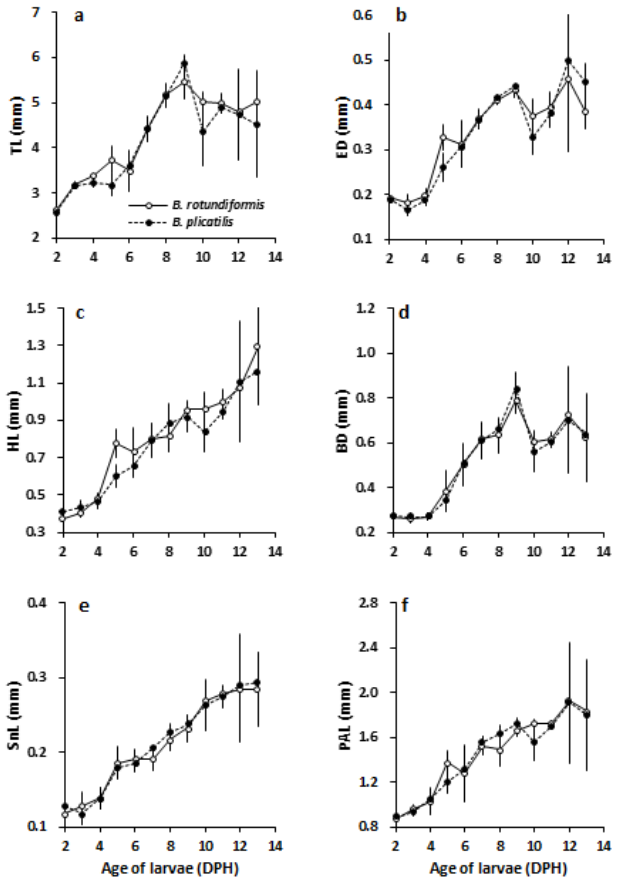

Fig. 5. Morphometrics of first-feeding silver therapon *Leiopotherapon plumbeus* larvae fed *B. rotundiformis* (open circles) or *B. plicatilis* (closed circles) at first feeding. (a) TL, total length; (b) ED, eye diameter; (c) HL, head length; (d) BD, body depth; (e) SnL, snout length; (f) PAL, pre-anal length. Open and closed circles indicate mean ± standard deviation, n = 10.

silver therapon larvae and afterwards with *Artemia* from 14 to 35 DPH. Results of this study will help advance the production of sufficient seedstock for possible culture and wild stock rehabilitation of this important food fish species. Further studies are needed to confirm these findings and to examine the feeding regimes for older silver therapon larvae and their effects on larval performance and composition when co-fed live and artificial diets.

ACKNOWLEDGEMENT

This study was funded by the SEAFDEC Aquaculture Department (Nr-01-F2013B). Thanks are due to N.B. Olorvida for assistance in the production and maintenance of the larvae.

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