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# MORPHOLOGICAL DEVELOPMENT AND SURVIVAL OF PHILIPPINE SILVER THERAPON LARVAE, *Leiopotherapon plumbeus* (Kner, 1864) REARED UNDER DIFFERENT FEEDING SCHEMES

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Philippine silver therapon, *Leiopotherapon plumbeus*, locally known as *ayungin*, is an endemic fish species in the country. The demand for silver therapon remained high despite the decline of its population and commercial catches. Taking into account the potential of this species for stock enhancement, this study focused on developing an initial feeding strategy necessary to provide potential *L. plumbeus* juveniles for aquaculture. The experiment conducted was a short duration 10-day feeding experiment which used different feeding schemes utilizing live food organisms [*Brachionus* (B), *Moina* (M), co-fed with BM, *Chlorella* (C) and filtered lake water (L)]. Morphometric measurements were evaluated to assess growth performance of larvae among different feeding schemes. Survival rate was determined at the end of feeding experiment at 9 DAH (days after hatching).

Growth parameters of *L. plumbeus* larvae reared under different feeding schemes showed significant differences starting at 5 DAH ($P < 0.05$) onward. After a total of 10 days, a significant decline in fish larval samples was observed under two feeding schemes (C and L). BM has a significant higher rate of survival (18.07 ± 7.09%) compared to other treatments. *B. rotundiformis* (15-20 $mL^{-1}$) can be considered as a starter food for silver therapon larviculture and *M. micrura* (5-10 $mL^{-1}$) can be utilized during an assumed co-feeding phase at 6 DAH.

Key Words: *Leiopotherapon plumbeus*; growth; first feeding; hatchery-bred; larvae

# 1. INTRODUCTION

## 1.1 Fish Production in Laguna de Bay

Laguna de Bay (14°10'-14°35' N, 121°-121°30' E) is the largest freshwater lake in the Philippines with an average surface area of 900 km$^2$. With such immense surface area, the fishery resources of the lake are considered to be one of the richest (Fortes, 1993). The lake is traditionally used for livelihood as supported by the earliest documented studies mainly focusing on fisheries (Mane and Villaluz, 1939; Villadolid, 1934). Delmendo and Bustillo (1968) documented that within 1960 to 1964, the fish population of the lake comprised of 23 species of fish belonging to 16 families and 19 genera. The study also noted that *Leiopotherapon plumbeus* and *Glossogobius giurus* were considered to be the important and dominant species of fish for commercial catches.

## 1.2 Distribution of Silver Therapon

Philippine silver therapon, *Leiopotherapon plumbeus* (Kner, 1864), locally known as *ayungin*, is an endemic fish species in the country (Mane, 1934) and is extensively distributed throughout Luzon. It was considered to be the most abundant fish in Laguna de Bay until 1991 and it comprised approximately more than 70% of the fish fauna found in the lake (Mercene and Cabrera, 1991). However, there was an observed substantial change in species composition and relative abundance of fishes found in the lake (Palma *et al.*, 2002; Mercene, 1987). In particular, a declining trend in the population of silver therapon has been recorded in 2002 (Palma*, et al.*, 2002) ranking this indigenous species to be only third in terms of abundance. According to a report released by the Bureau of Agricultural Statistics (BAS, 2002), fish production of *L. plumbeus* in a 2001 catch survey was merely 3 070 metric tons of the recorded 62 163 metric ton total inland municipal fish production. These changes in species diversity may be attributed to several interrelated factors including rapid deterioration of lake water quality due to domestic and industrial pollution in addition to unintentional species introductions and stocking for fishery enhancement.

## 1.2 Captive Breeding of Silver Therapon

According to reports of the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), the demand for silver therapon remained high despite the decline of its population and commercial catches within Laguna de Bay. With that, silver therapon shows high potential for aquaculture as a food fish and for stock enhancement. To address this concern, several projects on captive breeding and larval rearing has been done (PCAARRD-DOST and UPLB-LRS, 2012 and SEAFDEC-BFS, 2015) which presented technologies on induced spawning, hatchery, nursery and larval rearing. In spite of the preliminary success in artificial propagation and rearing of silver therapon (PCAARRD-DOST, 2012), reliable and consistent rearing methods particularly success in initial feeding are still in their infancy. During the early larval stages, the transition from endogenous to exogenous feeding has been recognized as a significant cause of early mortality (Busch, 1996) creating a distinct gap in recent approaches in *L. plumbeus* farming.

## 1.3 Live Feeds and First Feeding

Live feeds are considered as the primary item in the diet of cultured fish larvae. During the first feeding phase, digestive tract is still rudimentary and digestion process occurs in the hindgut epithelial cells (Govoni, Boehlert and Watanabe 1986). Such developing digestive system is still incapable of processing formulated diets, thus larvae are fed live feeds permitting growth and survival. Even with recent progress in the improvement of inert diets for fish larvae, (Cahu and Infante 2001) feeding of most potential species for commercial aquaculture still relies on live feeds on the duration of early ontogenetic development.

Rotifers are the most important live food organisms for use as starter food for rearing small fish larvae (Divya et al., 2011; Shiri Harzevili et al., 2003; Lubzens et al., 2001). Brackish and saline rotifers in particular are considered as imperative live food organisms for use in tropical aquaculture due to their smaller lorica length applicable to fish species with small mouth openings (Lim et al., 2003; Lubzens et al., 2001).


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Despite the fact that the more popular species of zooplankton utilized for fish seed production are rotifers, the potential of the freshwater flea, *Moina sp.* as larval food has also received considerable attention (Erman, 1956). Aquaculturists also accentuate the value of cladocerans as food for the young and adult stages of various fishes. *Moina sp*. has been expansively employed as prey for the maintenance and production of fish species of commercial value (Fermin and Bolivar, 1991; Free, 1966; Cooper and Grasslight, 1963). Studies showed that *Moina sp*. significantly improve growth and survival of marine and freshwater fish species (Fermin and Bolivar, 1991; Villegas, 1990; Fermin and Recometa, 1988; Baldia *et al*., 1985).

Taking into account the potential of this species for stock enhancement, this study focused on developing an initial feeding strategy necessary to provide potential *L. plumbeus* juveniles for aquaculture. The experiment conducted was a short duration 10-day feeding experiment which used different feeding schemes utilizing live food organisms including *Brachionus rotundiformis* and *Moina micrura*.

## 2. METHODOLOGY

Mass culture procedures, induced breeding routines and feeding experiments were all done at Southeast Asian Fisheries Development Center-Aquaculture Department, Binangonan Freshwater Station (SEAFDEC/ AQD-BFS).

### 2.1 Mass Culture of Live Food Organisms

A progressive batch culture technique suggested by Southern Regional Aquaculture Center (Creswell, 2010) was adapted for the mass production of *Chlorella ellipsoidea*. Algal density was kept standardized during feeding of prey at a density of $10^5$ to $10^6$ cells $mL^{-1}$, *C. ellipsoidea* was harvested during the exponential growth phase and density was checked using a hemacytometer cell count chamber. Zooplankton were mass cultured using techniques adapted from Lavens and Sorgeloos (1996). Upscaling of rotifer *B. rotundiformis* and *M. micrura* started using 1 000 mL erlenmeyer flasks maintained under laboratory conditions. Starter cultures were supplied with *C. ellipsoidea* at approximately 300 mL of algae added daily. Temperature in the culture containers ranged from 28-30°C and were maintained at a 1:1 light and dark photoperiod.

### 2.2 Induced Breeding

Parental fishes were selected for the breeding set-ups and initially placed in separate male and female maintenance tanks (approximately 30L). Parental fishes were acclimatized for 24 hours prior to hormone administration. Hormone was administered at a concentration of 50IU/g body weight using a 26 gauge needle with 50% of the total volume injected in each lateral side of the fish.

### 2.3 Larval Rearing

Newly-hatched larvae were all obtained from a single batch of parental fishes and were randomly transferred to mildly aerated rearing aquaria (15L) with a stocking density of 15 larvae $L^{-1}$. First feeding experiment included a 10-day short duration feeding experiment from 0 DAH to 9 DAH. Feeding experiments were performed under different feeding conditions: (1) rotifer *B. rotundiformis* was provided all throughout the rearing period (*B*); (2) *M. micrura* was provided during the entire rearing period (*M*); (3) only algae *C. ellipsoidea* ($10^5$ to $10^6$ cells $mL^{-1}$) was provided throughout the rearing period (*C*); (4) filtered lake water with wild zooplankton and phytoplankton (*L*); (5) co-feeding treatment using *B. rotundiformis* and *M. micrura* (*BM*). Prior to stocking of larvae, rearing water was pre-conditioned for 24 hours by integrating 5L of *C. ellipsoidea* ($10^5$ to $10^6$ cells $mL^{-1}$) except for treatment supplied with lake water. The administration of live food started at 0 DAH at 08:00, 14:00 and 20:00 hour. The concentration of zooplankton was determined through volumetric counts using means of three replicates in 1mL samples. Feeding amount was adjusted daily according to the desired prey densities in each tank. All treatments were carried out in four replicates each in a completely randomized design.

### 2.4 Data Collection and Statistical Analysis

Nine larvae were sampled daily to evaluate growth performance of larvae. Total length (TL) was measured from the tip of the snout to the posterior margin of the body. Pre-anal length (PL) was measured from the tip of the snout to the anus. Body depth (BD) referred to the height of the body immediately posterior to the anus. Eye diameter (ED) was measured using the orbital margin before pigmentation started. Gape size (GS) was measured by following Shirota (1970) wherein, GS = $\sqrt{2}$ *x* upper jaw length (UJ). Growth of larvae and juveniles was assessed in terms of specific growth rate (SGR). The following growth parameter was calculated as adapted from (Chen et al., 2006):


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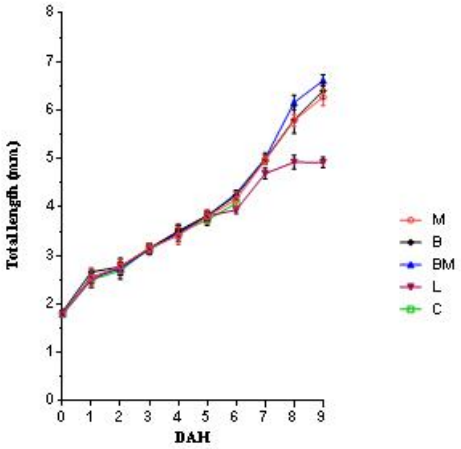


Fig. 1. Comparison of TL among treatments

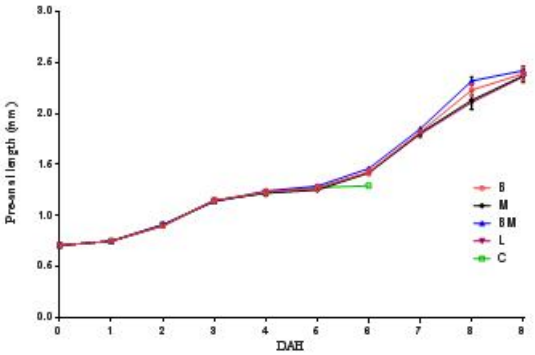


Fig. 2. Comparison of PL among treatments

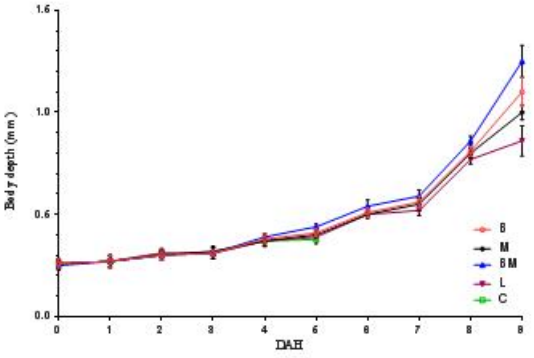


Fig. 3. Comparison of BD among treatments

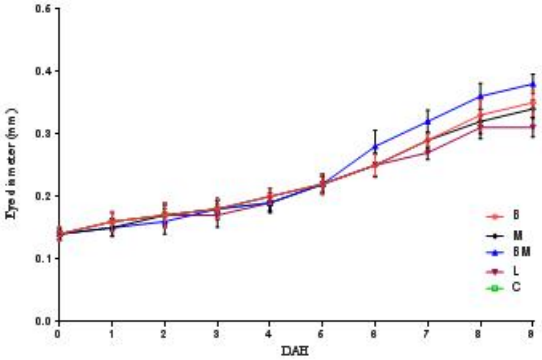


Fig. 4. Comparison of ED among treatments

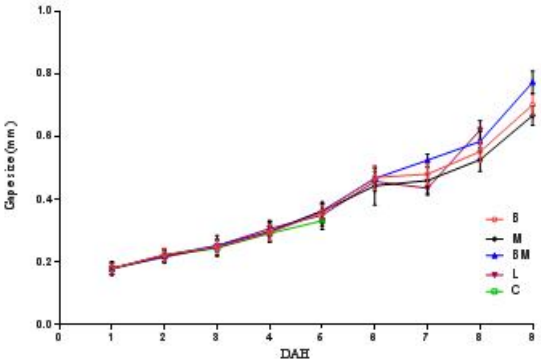


Fig. 5. Comparison of GS among treatments

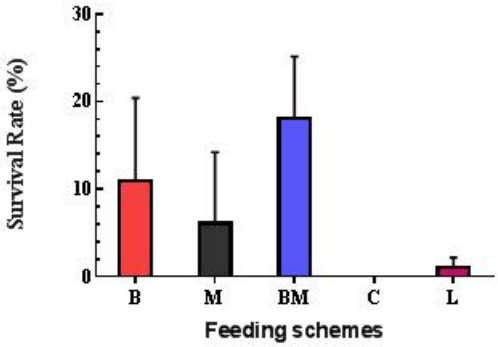


Fig. 6. Comparison of SR among treatments


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SGR (%/day) = $100(\text{Ln}T_{Lf} - \text{Ln}T_{Li}) / \Delta t$, where $T_{Lf}$ and $T_{Li}$ are the final and initial total length, respectively and $\Delta t$ is the time interval (days) between sampling days. Survival rate of larvae was assessed after 9 DAH. Morphometric measurements (n = 9, mean ± SD) observed under different feeding schemes were compared using One-way ANOVA ($P < 0.05$). All analysis and graphs were created using GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego California USA, www.graphpad.com).

## 3. RESULTS AND DISCUSSION

A total of nine parental fishes, at a ratio of one female to two males per tank were subjected in every induced spawning routine. Water quality parameters including water temperature (26.63 ± 1.98 ºC), dissolved oxygen (7.19 ± 0.35 $mgL^{-1}$) and pH level (8.55 ± 0.10) were at ambient conditions during the induced breeding procedures. Spawning took place approximately 26-36hrs (30 ± 3.09 hrs) after injection at 26.5 ± 1.41 ºC. Immediately after spawning, collected egg samples have a diameter range of 0.32-0.60 mm (0.38 ± 0.01mm). Fertilized eggs hatched 16-22 hours (19 ± 2.53 hrs) after spawning at 26.9 ± 1.13 ºC.

At early developmental stages particularly at the yolksac stage, *L. plumbeus* larvae showed a rapid increase in TL. As shown in figure 1, larvae with TL in between 1.6 mm to 2.0 mm also confirmed a quick resoprtion of yolk. Thereby, such rapid increase in TL is associated with quick yolk utilization (Williams *et al.*, 2004). Nonetheless, no significant difference in TL among treatments was observed during the endogenous feeding stage. Larvae co-fed with *Brachionus* and *Moina* (BM) showed higher increment in TL at 9 DAH (6.61 ± 0.12); thereby, presenting as well a significant increase in SGR (Table 1). *Brachionus sp.* resulted to significant accelerated fish larval growth when fed with algae in *Amphipron sebae* (Divya *et. al*, 2011) and *Lota lota* (Harzevili *et al.*, 2003).

Table 1. Specific growth rate of *L. plumbeus* larvae

| Feeding Schemes | Initial Length (mm) | Final Length (mm) | SGR (% $day^{-1}$) |
|---|---|---|---|
| B | 0.60 ± 0.03 | 1.86 ± 0.03$^{a}$ | 13.84 ± 0.43$^{a}$ |
| M | 0.59 ± 0.03 | 1.84 ± 0.03$^{ab}$ | 13.89 ± 0.53$^{a}$ |
| BM | 0.59 ± 0.02 | 1.89 ± 0.02$^{ac}$ | 14.40 ± 0.32$^{a}$ |
| C | 0.58 ± 0.04 | 1.41 ± 0.01$^{d}$ | 9.14 ± 0.48$^{b}$ |
| L | 0.59 ± 0.03 | 1.59 ± 0.02$^{e}$ | 11.10 ± 0.38$^{c}$ |

Mean ± SD with different superscript letters indicate significant difference ($P < 0.05$)

Mean values of PL showed significant difference among five feeding schemes at 6 to 8 DAH ($P < 0.05$). Except for treatment C, a consistent increase in PL is observable until 8 DAH (Figure 2). Increase in PL significantly denotes development in the head region including formation of a more defined upper and lower jaw (Ogata *et al.*, 2010) and otic capsules (Aya *et al.*,2015). Also, the anal orifice shifted posteriorly providing an additional increased in PL proportion. Conversely at the end of the first feeding experiment, observed PL showed no significant difference among treatments ($P < 0.05$). Mean values of PL among four treatments (B, M, BM and L) measured ca. 39% ± 0.026 of TL at 9 DAH. This particular period may suggest the initial constant growth in *L. plumbeus* body proportion similar to other related species (*A. sebae*, Divya *et. al*, 2011; *H. malcolmi*, Ogata *et al.*, 2010; *A. testudineus*, Morioka et al., 2009; and *L. lota*, Harzevili *et al.*, 2003) regardless of of prey type available.

Yolksac larvae have a mean BD of 0.258 ± 0.002 mm. which is approximately 14.23% of the initial TL. A continuous gradual increase in BD can be observed from mean values of *L. plumbeus* larvae under different feeding schemes (Figure 3). Observed change in BD may be attributed to yolk resorption and body extension in preflexion larvae. Significant difference in BD among treatments was already noted at 5 DAH ($P < 0.05$). Similarly, all treatments were significantly different from each other at 9 DAH. Treatment co-fed with *Brachionus* and *Moina* has the highest significant increase in BD at 9 DAH ($P < 0.05$) which was also parallel to the observed significant increase in TL at the BM set-up. BM has an observed 1.25 ± .078 mm BD at the last day of first feeding experiment. Conversely, L has the lowest measured BD (0.86 ± 0.07 mm) among treatments at 9 DAH.

Initial measurement of ED (0.142 ± 0.002 mm) among treatments did not differ significantly during the yolksac stage at 0 DAH.


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However, significant difference ($P < 0.05$) was already noted in ED at 6 DAH. BM has the highest significant increase in ED with 0.28 ± 0.026 mm at 6 DAH but treatments B (0.25 ± 0.017 mm), M (0.25 ± 0.019 mm) and L (0.25 ± 0.018 mm) has no significant difference ($P < 0.05$). Noted change in ED is mainly attributed to orbital development. Such development is also parallel with the start of pigmentation in the orbital area between 18 to 24 HAH.

Development of mouth morphology significantly contributes in first feeding ability of larvae (Busch, 1996; Johnston, 1993; Collins and Nelson, 1993). Newly hatched *L. plumbeus* larvae began to establish defined maxillary structures only at 1 DAH though were still non-functional. Similar to observation published by PCAARD-DOST (2012), mouth parts of *L. plumbeus* larvae started to become functional at 3 DAH which was closely related to the observed time for complete resorption of yolk and oil globule reserves. Gape size (GS) as predicted using the lower jaw length showed gradual increase in size during the first feeding experiment. Initial measurement of functional GS in newly-hatched larvae at 3 DAH was 0.249 ± 0.003 mm with no significant difference among treatments. It was in 7 DAH that a significant difference was noted among treatments B, M, BM and L ($P < 0.05$).

Indoor rearing of hatchery-bred *L. plumbeus* larvae fed under different feeding schemes obtained low survival rates. Among the five feeding schemes utilized, BM has a significant higher rate of survival (18.07 ± 7.09%) compared to other treatments (Figure 6). Conversely, survival rates obtained from other treatments (B, M, C and L) were not considered significantly different ($P < 0.05$).

## 4. CONCLUSION

This study aims to provide basis for initial feeding technique of *L. plumbeus* larvae utilizing cultured live food organisms as prey. Growth performance and survival of larvae reared under different feeding schemes has been affected by the different prey items offered. Treatment co-fed with *B. rotundiformis* and *M. micrura* showed improved morphological development compared to treatments singly fed with other live food organisms. *B. rotundiformis* is an ideal food item for initial feeding of *L. plumbeus* larvae followed by *M. micrura* offered at 6 DAH.

For further improvement, this study suggests the use of enrichments for cultured zooplankton, thus may affect growth and survival of larvae.

## 5. ACKNOWLEDGMENTS

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