Survival, condition factor and growth performance of endemic silver therapon *Leiopotherapon plumbeus* (Perciformes: Terapontidae) reared in varying salinities

[1,2]Mark N. C. Corpuz, [1,2]Nikki B. C. Roque, [1]Adrian D. C. Manliclic

[1] Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, Bayan, Bataan, Philippines; [2] Graduate School, School of Environmental Science and Management, University of the Philippines Los Baños, Los Baños, Philippines. Corresponding author: A. D. C. Manliclic, deilmanliclic@gmail.com

Abstract. Silver therapon (*Leiopotherapon plumbeus*, Kner 1864) is an endemic fishery resource with a significant contribution to the subsistence fisheries and livelihood of local fishing communities. The present study evaluated the survival, condition factor and growth performance of acclimatized *L. plumbeus* cultured in varying salinities under indoor aquarium set-up. A total of 240 *L. plumbeus* specimens (weight=0.119±0.003 g; TL (total length)=22.352±0.451 mm), without sex distinction, were randomly distributed in four salinity treatment groups (0, 10, 20, and 35‰), with three replicates per treatment. Salinity significantly affected the survivability of the experimental fish, with the lowest mean survival rate being observed in 35‰ salinity. After 42-day culture period, fish body weight and feed conversion ratio were statistically homogenous among treatments (p>0.05). Condition factors were below the ideal score of 1.00, albeit the lowest value was recorded in 35‰ salinity (K=0.71). The highest condition factor and growth coefficient (b) scores were observed in 10‰ salinity. This preliminary study showed the wide salinity tolerance of acclimated *L. plumbeus* and the capacity of the population to survive and grow even at full-strength seawater.
Key Words: Ayungin, Bataan, brackishwater, L-W relationship, Orani.

Introduction. The continuous need of freshwater for domestic and agro-industrial development restraints the expansion of freshwater aquaculture, particularly in groundwater-dependent and rain-fed areas at the tropics (Phillips et al 1990; Okello et al 2015). This plight emphasizes the pressing need for an efficient use of water from saline habitats (e.g., brackishwater) as alternative culture environments for fish farming (Verdegem & Bosma 2009). In the Philippines, one of the main strategies for an increased overall fish production is by enhancing the aquaculture productivity in brackishwater areas (Stevenson et al 2005). The goal led to the creation of salt-tolerant aquaculture species (Tayamen et al 2004), and to the application of verification trials for the refinement of their culture protocols, under saline conditions (Rosario et al 2004; Jaspe & Caipang 2011).

Understanding the influence of environmental stressors is one of the critical challenges for an efficient and sustainable aquaculture management (Boyd & Tucker 2012). Among the water quality variables, environmental salinity has been widely recognized as a major determinant affecting the survival (Guo et al 1995; Manliclic et al 2018), growth (Boeuf & Payan 2001; Imsland et al 2001) and reproduction (Guo et al 1993; Haddy & Pankhurst 2000) of several commercially important fishes. Furthermore, the effects of salinity in aquaculture must be thoroughly understood as salinization in freshwater habitats could be inevitable due to the climate change and seawater intrusion (Venâncio et al 2020).

Among the finfishes in the Philippines, silver therapon (*Leiopotherapon plumbeus*, Kner 1864) is being considered as a good candidate for aquaculture (Aya 2019): it is a major fishery resource supporting the subsistence fisheries and livelihood in local fishing communities (Aya 2021; Corpuz & Espaldon 2021). This endemic terapontid can be bred in captivity using hormonal induced spawning (Consigna et al 2019) and shows promising characteristics ideal for aquaculture ventures (Pillay & Kutty 2005). Wild populations of *L. plumbeus* are known to occur in several freshwater bodies in Luzon Island, Philippines (Corpuz et al 2016; Paller et al 2017; Corpuz 2018). Studies on hatchery management (Aya et al 2014; Añano et al 2015), early development (Aya et al 2017), population ecology (De Leon et al 2017; Santos et al 2020), and acute salinity tolerance (Corpuz & Manliclic 2020) of *L. plumbeus* have been reported. However, little is known on how environmental salinity affects the acclimated *L. plumbeus* under indoor semi-controlled conditions. As such, the present study investigated the influence of different salinity levels on the survival, growth, condition factor and feeding performance of the hatchery-bred juvenile *L. plumbeus*.

Material and Method

Experimental fish. Three hundred sixty unsexed juvenile *L. plumbeus* (30 days after hatching) with an initial length of 15–25 mm total length (TL) and an average weight of about 0.1 g were randomly obtained from the parental stock that are being maintained in the hatchery units of the Bataan Peninsula State University – Orani Campus. Disease was not detected in any of the fish samples prior to stocking.

Experimental set-up. The study utilized four salinity treatments (0, 10, 20 and 35‰ or g L^{-1}) assigned in a completely randomized design, with three replicates for every treatment. Twelve aquaria (20 L capacity) were used as rearing compartments.

Preparation of seawater. Artificial seawater was prepared based on the formulation used by the Southeast Asian Fisheries Development Center/Aquaculture Department, as reported by Corpuz & Manliclic (2020). The artificial seawater was aerated using a root blower for three days to eliminate the chlorine. Salinity of media for the treatments were monitored using a salinometer.

Acclimation. The *L. plumbeus* were acclimated in 30 L basins gradually, from freshwater until 35‰ salinity, over seven days, i.e., one day acclimation for each desired treatment (from an intermediate salinity directly to the full sea water salinity). Fish were fed twice a day (09:00 and 15:00 h) with egg custard and bloodworm diets until apparent satiation. The experimental units were supplied with mild artificial aeration, net enclosures, and reared under 1:1 h (day:night) photoperiod regime. In case of fish mortalities of more than 10, additional fish were immediately replaced to compensate the reduction, following the gradual acclimation procedure.

Experimental system. After 7 days of acclimation, 240 unsexed *L. plumbeus* (weight: 0.119±0.003 g; TL: 22.352±0.451 mm) from the acclimation basins were designated to the four different treatments in a 20-L aquarium (stocking density of 1 fish L^{-1}). All aquaria were covered by net enclosures throughout the experiments to prevent the fish from escaping. The experimental units were provided with artificial aeration, regular cleaning and water exchange (2 L daily and 15 L weekly) to compensate for evaporation. Salinity levels were regularly adjusted accordingly. The net enclosures were washed and cleaned once a week during the sampling. The light regime was maintained at a 1:1 h day and night photoperiod. Feeding management followed the protocol during acclimation; pelletized commercial feeds (32% crude protein) were given in the last two weeks of rearing. Water quality parameters including temperature (°C), dissolved oxygen (mg L^{-1}) and salinity (‰) were monitored twice a day at 09:00 and 15:00 h. Levels of pH and TAN NH_3^- (total ammonia nitrogen, in mg L^{-1}), and NH_4^+ (mg L^{-1}) were determined weekly using the Koroleff's Indophenol Blue Method (APHA 1998). Sampling was carried

out in the 2nd, 4th and 6th week of culture. Feeding was ceased 24 h prior to sampling. Measurement of TL was done in the first and last sampling using a digitized Vernier caliper. Fish body weight was determined using an electronic balance.

Survival and growth performance. Survival and growth performance were evaluated after 42-day rearing period. The fish survival rate in each treatment was determined using the formula, following Stejskal et al (2021):

$$S (\%) = (n_f / n_i) \times 100$$

Where:
n_f - number of living fish at the end of the experiment;
n_i - initial number of fish stocked.

The fish body weight was measured, including the initial mean weight (preliminary bulk weight per treatment) and final mean weight (total body weight/final number of fish per treatment), mean gain in weight (difference between the final mean weight and the initial mean weight).

The specific growth rate (SGR) was computed following the approach of Stejskal et al (2021):

$$SGR \% \ day^{-1} = \frac{(Ln\ Wt - Ln\ Wo)}{t} \times 100$$

Where:
Ln - natural logarithm;
t - experiment duration (day);
Wt - initial weight;
Wo - final weight.

The survival rate is the ratio between the final number of fish and the initial number of fish, multiplied by 100. The FCR and FCE were computed as follows (Pillay & Kutty 2005):

$$FCR = F / (Wt-Wo)$$

Where:
F - the amount of given feed;
Wt - initial weight;
Wo - final weight.

$$FCE (\%) = (1 / FCR \times 100)$$

Length-weight relationship and condition factor. For length and weight relationship (LWR), the total length was measured (to the nearest 0.01 mm) using a Vernier caliper. Wet weight of fish (W) was determined using a digital balance (g). The allometric relationship between length (L) and weight (W) was calculated by the formula, following Ricker (1973):

$$W = a\ L^{\ b}$$

Where:
W - weight of an individual fish (g);
L - length of an individual fish (mm);
a and *b* - constants.
    The data of total length and weight will be analyzed by the least square method using the equation of Le Cren (1951) given as:

$$Log\ W = Log\ a + b\ Log\ L$$

Where:
a and b - constants estimated by linear regression of the log transformed variates.

The LWR was determined separately for each treatment after 42 days of culture. The fish body condition factor (K) was calculated using the function (Le Cren 1951):

$$K = \frac{W}{a\,TL^b}$$

Where:
K – condition factor;
W - fish weight (g);
TL - total length (mm);
a - regression intercept;
b - regression slope.

Data analyses. Homoskedasticity (Levene's test) and normality (Shapiro-Wilk test) of variances were tested for growth and water parameters. All of the variables met the assumptions and were subjected to univariate parametric tests. Growth and length-weight data were analyzed and compared among treatments (mean ± SD) using one-way analysis of variance (ANOVA) ($p < 0.05$). Survival data were arc-sined for parametric analysis ($P < 0.05$). Pairwise comparison between treatments was determined using Tukey's Honestly Significant Difference Comparison Test ($p < 0.05$).

Results

Water quality parameters. Summary of water quality variables is presented in Table 1. Mean temperature, DO and pH were significantly homogenous among the treatments ($p > 0.05$). Variations in mean TAN (NH_3^-, NH_3-N, NH_4^+) were found significant among treatments ($p < 0.05$), with 35‰ having the highest TAN readings as compared to other treatment groups.

Table 1

Mean ± standard deviation of water quality parameters in different salinity levels for the whole culture period

Water quality variables	Mean ± SD				F value
	0‰	10‰	20‰	35‰	
Temperature	26.83±0.06[a]	26.58±0.75[a]	26.66±0.81[a]	26.73±0.15[a]	1.28[NS]
DO	5.55±0.62[a]	5.43±0.68[a]	5.41±0.70[a]	5.36±0.71[a]	3.25[NS]
pH	8.57±0.09[a]	8.47±0.10[a]	8.43±0.16[a]	8.19±0.15[a]	1.87[NS]
NH_3-	0.03±0.08[a]	1.19±0.26[b]	2.16±0.29[c]	2.65±1.69[d]	65.50**
NH_3-N	0.03±0.01[a]	1.65±0.32[b]	2.48±0.53[c]	3.45±2.25[d]	523**
NH_4	0.04±0.01[a]	1.05±0.48[b]	2.84±0.42[c]	3.17±1.88[d]	467**

[*] Significant at $p < 0.05$; [**] Significant at $p < 0.01$; [NS] Not Significant at $p > 0.05$. Row with similar letter indicates no significant difference.

Survival rate. Varying environmental salinity had a significant effect on fish survival ($F = 5.63$, $p < 0.05$). The lowest survival rate (63.33±10.41%) was observed for 35‰, and found to be statistically different among all other treatments ($p < 0.05$). While treatment at 20‰ exhibited the highest survival rate (88.33±7.64%), this observation did not significantly vary between salinity treatments at 0‰ (85.00±8.66%) and at 10‰ (81.67±6.67%) ($p > 0.05$).

Growth performance. All treatments exhibited an apparent increase in the mean body weight, after a 42 days culture period (Table 2 and Figure 2). Although the highest values were observed in treatment at 10‰ salinity, all growth variables were statistically homogenous among treatments ($p > 0.05$) (Table 2). Overall, the final body weight

(0.216±0.13 g) was significantly higher than the initial body weight (0.119±0.011 g) (p<0.05). The levels of FCR increased with increasing levels of salinity, albeit the variations among treatments were not significant (p>0.05).

Mean ± standard deviation of growth parameters of *Leiopotherapon plumbeus* reared in varying salinity levels

Growth variables	Mean ± SD				F value
	0‰	*10‰*	*20‰*	*35‰*	
Initial weight (g)	0.128±0.004[a]	0.114±0.016[a]	0.119±0.009[a]	0.116±0.012[a]	1.006[NS]
Final weight (g)	0.221±0.014[a]	0.227±0.004[a]	0.209±0.01[a]	0.207±0.015[a]	2.198[NS]
Gain in weight (g)	0.093±0.012[a]	0.113±0.019[b]	0.09±0.013[ab]	0.091±0.01[ab]	1.907[NS]
Daily gain in weight (g)	0.002±0.001[a]	0.03±0.001[a]	0.002±0.001[a]	0.002±0.001[a]	1.79[NS]
Specific growth rate (% day $^{-1}$)	1.295±0.124[a]	1.663±0.38[a]	1.337±0.216[a]	1.379±0.15[a]	1.458[NS]
Food conversion ratio	3.56±1.02[a]	3.59±0.86[a]	3.73±0.62[a]	4.18±1.14[a]	2.85[NS]

[*] Significant at p<0.05; [**] Significant at p<0.01; [NS] Not Significant at p>0.05. In a row, similar letter indicates no significant difference.
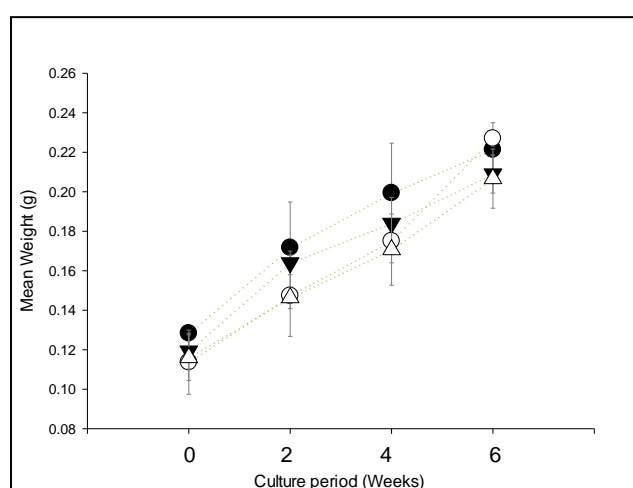

Figure 2. Changes in mean weight of *Leiopotherapon plumbeus* during the 42-day culture period under varying salinities. 0‰ (●); 10‰ (○); 20‰ (▼); 35‰ (Δ). Vertical bar=SD.

Condition factor and length-weight relationship. The K and LWR of the fish samples reared in different environmental salinities are presented in Table 3. The standard equation followed by the regression curve shapes was log10 weight=*a*+*b* (log10 Length). The K values in all treatments were under the ideal score of 1.0. The results correspond to the observed negative allometric growth as inferred from the values of slope (*b*), which were inferior to 3.0 for all the treatments.

Condition factor and length-weight regression coefficients for juvenile *Leiopotherapon plumbeus* reared in different salinities, after 42 days of culture

Treatments	n	K	a	b	R^2	P
0‰	51	0.848	0.053	2.192	0.881	<0.021
10‰	49	0.946	0.034	2.614	0.913	<0.027
20‰	53	0.904	0.029	2.561	0.842	<0.023
35‰	38	0.710	0.057	1.769	0.822	<0.022

Discussion. The rearing conditions were within the acceptable ranges (Boyd & Tucker 2012), except for the mean values recorded in NH_3^-, NH_3^-N and NH_4^+. Uneaten feeds and net metabolites of fish were contributory factors in the increased TAN, and this was directly associated with increasing salinity levels. As salinity increases, aquatic species tend to spend more energy on osmoregulation to maintain the ionic and osmotic balance between their bodies (plasma) and the surrounding environment (Kültz 2015). Various osmoregulatory mechanisms (e.g., increased drinking rate) require considerable energy and fish could prioritize certain metabolic processes to compensate the increased energy demand. The elevated ammonia levels at increasing salinities were likely results of protein catabolism which tends to compensate the increase in energy demand, in an attempt to maintain the ionic and osmotic balance (Ballantyne 2001). In *Dicentrachus labrax*, a euryhaline teleost, the hyperosmotic condition demanded more energy to cope with the environment than extreme hypoosmotic conditions (Ordóñez-Grande et al 2021). The increasing ammonia levels could also be related to the deamination of amino acids during energy production (Aragão et al 2008).

The present findings indicated the wide-range salinity tolerance of fish when exposed to freshwater and polyhaline salinity levels following a gradual acclimation. Varying salinity levels have no profound effect in body weight variables among treatments, albeit the fish survival rate in marine conditions was negatively affected. In a recent work, direct immersion in a 35‰ saline environment resulted into a 50% mortality rate for a 96h exposure time (Corpuz & Manliclic 2020). A closely related *Bidyanus bidyanus* was able to tolerate a 12 ppt salinity with 100% survival rate, although the mortality progressively increased with increasing salinity (Guo et al 1995). Similar observations were also found when the fish were hatched at varying salinities. Better survival of *B. bidyanus* juveniles hatched in saline environment (6 ppt) were obtained than for those hatched in freshwater. On the other hand, no larvae hatched when the salinity exceeded 18 ppt (Guo et al 1993). The present finding supports the view that a higher survival of *L. plumbeus* is attainable when introduced in water with a gradual increment of salinity rather than with abrupt changes (Corpuz & Manliclic 2020). Better survival rates are common in a number of aquatic species in presence of a slow increase of salinity over time, which increases the chances of survival in the aquatic environment, compared with abrupt changes (Kefford et al 2004).

The highest values of condition factor (K), growth (b) and regression coefficients (R^2) were found in 10 and 20‰ saline waters (Table 3). These scores were lower in comparison to the wild-caught specimens observed by De Leon et al (2017) and Santos et al (2020). The observed K value of less than 1.0 indicates that the fish specimens were exposed to unfavorable rearing conditions. Also, the negative allometric growth rate suggests that the fish specimens tend to become thinner and have reduced body weights in relation to their length. Exposure to low-salinity waters exhibited better K than for 35‰ saline waters. It is hypothesized that the fish osmoregulatory functions were improved in salinity levels similar to the brackish waters, compared to freshwater and marine environments. Energetic cost in mesohaline salinity is lower and the fish have an adequate energy budget for their growth (Boeuf & Payan 2001). The energetic cost of ion regulation is lower in an isotonic environment, where ionic gradients between blood and water are minimal, and the substantial energy savings increase fish growth (Morgan & Iwama 1991). The results are in line with Gutt (1985), who stated that the better growth of juvenile European flounder (*Platichthys flesus*) is in the middle of the salinity range (5-15‰). A number of fish species, often juveniles, were also reported to grow optimally in intermediary salinity conditions of brackish waters (Boeuf & Payan 2001).

The culture in marine salinity can suppress the conversion of feed into body mass in *L. plumbeus*. Similar observations were reported in red tilapia (Watanabe et al 1993) and puffer fish (Yan et al 2004), in which the feeding efficiency of the test specimens increased with lower salinity levels, whilst higher salinity levels generally adversely affected the feed intake. Imslands et al (2001) reported better growth and better food efficiency in turbot reared in intermediate salinities (15-19‰). Moreover, the highest feed consumption and feeding efficiencies were observed at salinities lower than full-strength artificial seawater. The present observation might be linked to the

osmoregulatory capacity of the aquatic organisms, in which the FCR increased because the feed intake was used to compensate the significantly higher iono- and osmoregulatory consumption at full strength seawater salinity (Boeuf & Payan 2001).

Conclusions. The present findings demonstrated the wide salinity tolerance of acclimated *L. plumbeus*, providing evidence of the euryhaline characteristics of this endemic terapontid. Varying environmental salinity levels had no pronounced effects on their growth, albeit better rearing condition can be achieved in lower salinity condition. Seawater salinity negatively influenced the fish survivability and condition factor under indoor set-up. This study provided important information for an improved aquaculture management of *L. plumbeus* as an alternative species for the brackishwater fish farming. Analysis of the influence of different concentrations of natural saline waters on the growth and metabolism of *L. plumbeus* should be performed in future studies. Moreover, chronic salinity tolerance tests for different life histories of *L. plumbeus* are highly recommended.

Conflict of interest. The authors declare no conflict of interest.

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Authors:
Mark Nell Castillo Corpuz, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines, e-mail: marknellcorpuz@yahoo.com
Nikki Bryce Cruz Roque, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines, e-mail: nbcroque@gmail.com
Adrian Deil Castro Manliclic, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines, e-mail: adcmanliclic@bpsu.edu.ph