Acute salinity tolerance of silver therapon, *Leiopotherapon plumbeus*

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Abstract. Despite the ecological and economic importance of silver therapon (*Leiopotherapon plumbeus* (Kner, 1864)), there is still no information regarding the salinity tolerance of this endemic freshwater terapontid. The present study evaluated the acute salinity tolerance of silver therapon. Salinity toxicity test (LC_{50} at 96 h) determined the toxicity limit of 315 unsexed *L. plumbeus* (4.39±0.39 cm) using seven salinity treatments (0, 7.5, 15, 20, 25, 30, and 35 g L^{-1}). Using the graphical method, LC_{50} projected salinity toxicity of approximately 29.92 g L^{-1}, albeit Probit analysis estimated a mean LC_{50} of about 30.70 g L^{-1}. After 96 h, 0, and 7.5 g L^{-1} salinity treatments obtained zero mortality, whilst 15, 20, and 25 g L^{-1} acquired < 7% mortality. Mortality rates of 51.11% and 62.22% were recorded at 24 h in treatments 30 g L^{-1}, and 35 g L^{-1}, respectively. Log-rank analysis of the Kaplan-Meier function determined the significant difference of sample survival times in different salinity concentrations (X^2 = 123.76), with an apparent decrease in survival rate that occurred in 30 g L^{-1} salinity. This preliminary study demonstrated that *L. plumbeus* can tolerate direct transfer to brackish water and can even survive in seawater.

Key Words: *Ayungin*, Bataan, brackishwater, LC_{50}, Probit analysis.

Introduction. Salinity is an important factor affecting the growth and survival of fish species (Barletta et al 2005). It is a significant water quality parameter for aquatic organisms since the osmotic effect influences their physiological condition, particularly osmoregulation and bioenergetics (Sterzelecki et al 2013). Through generating changes in the ecological factors and production of food organisms, water salinity affects fishes directly or indirectly. In recent studies, water salinity showed a significant impact on the survival of various fishes, especially during aquaculture operations (Iqbal et al 2012; Lisboa et al 2015; Manliclic et al 2018), and can even affect the competitive interactions between organisms (Alcatraz et al 2007). Salinity also serves as the physical barrier in the distribution and abundance of a wide array of marine and freshwater animals (Bringolf et al 2005).

Silver therapon, *Leiopotherapon plumbeus* (Kner, 1864) locally known as *ayungin*, *alukaok*, and *bagaong*, is an endemic freshwater species in the Philippines (Mane 1934). It is a native, freshwater, small silver-colored fish that eats a wide variety of organisms (Kock et al 2008). It is usually occurring in vegetated lentic environments and is known to occur in various freshwater bodies (Paller et al 2017; Corpuz et al 2016) and estuaries (De Leon et al 2017; Santos et al 2020) in Luzon. Locally, this species has high economic importance and demand due to its palatability, which caught the interest of fish farmers making it a potential candidate species for aquaculture (Aya et al 2015; Consigna et al 2019). However, its population is currently declining due to overexploitation, pollution (Guerrero 1996), and the presence of invasive fish (Corpuz 2018). As a result, this fish is now rarely seen in the local markets. Because of this scenario, the fish species is now identified as a target fish species for both aquaculture and conservation programs (Cruz 2009).

In Bataan, wherein the targeted cultured commodities are mangrove crab (*Scylla* spp.), prawn (*Penaeus monodon*), and milkfish (*Chanos chanos*), *L. plumbeus* is usually caught during harvest; this terapontid is considered feral and not intentionally stocked for brackishwater farming. Since the occurrence of *L. plumbeus* in the estuarine environment was just recently reported (De Leon et al 2017), the salinity tolerance of the fish is still unknown. Thus, the extent of the physiological limit of the downstream distribution and migratory capability, and the influence of salinity on the growth and survival of the fish also remain unknown. Although the salinity tolerance of the fish could be possibly estimated based on the salinity of the water where it was captured, the maximum salinity tolerance could not be accurately defined solely by field observations (Kefford et al 2004). No information regarding the estimated salinity tolerance (LC_{50} at 96 h) of this fish species was available in scientific peer-reviewed literature. To the best of our knowledge, this is the first attempt to evaluate the influence of salinity on the survival of *L. plumbeus*. Hence, this study determined the acute median lethal salinity concentration (LC_{50}) of the endemic *L. plumbeus* under a laboratory condition.

Material and Method

Experimental fish. Specimens of *L. plumbeus* were collected at brackishwater fishpond (salinity: 22.9±1.65 g L^{-1}) in Orani, Bataan (14°48’26” N, 120°32’40” E). Collected fish specimens were then transferred and conditioned in a concrete tank (25,000 L capacity) with a continuous flow of freshwater for six weeks. During conditioning, fish were fed daily in *ad libitum* with formulated pelletized feeds (crude protein = 42%).

Experimental set-up. After the conditioning, fish were transferred to glass aquaria (30 cm x 15 cm x 30 cm) filled with 15 L of de-chlorinated water. Each aquarium was provided with aeration and covered with a net. The fish were stocked at a rate of 1 fish L^{-1} and they were conditioned for another 48 h in the aquaria before applying the treatments. After the 48-h acclimatization, the salinity in the units was abruptly increased following the treatments. Seven salinity groups were utilized: 0, 7.5, 15, 20, 25, 30, and 35 g L^{-1}. Treatment groups were set in a completely randomized design with three replicates in each group. A total of 315 unsexed *L. plumbeus* (4.39±0.39 cm; 1.17±0.30 g) were utilized in this study. Artificial seawater was used in increasing the salinity in the treatments. It was prepared following the formulation used by the Southeast Asian Fisheries Development Center (Table 1). Salinity in the treatments was adjusted and monitored accordingly using a handheld refractometer (Atago hand refractometer). The *ad libitum* feeding regimen was continued during the experiment and the aquaria were cleaned regularly. The study was done in an indoor setting under an ambient culture condition (24-26°C) from February to April 2018.

Table 1

Composition of artificial seawater solution used in the study

Chemicals/Reagents	*30‰ (g 100 L^{-1})*
Sodium Chloride – NaCl	3,400.0
Magnesium Sulphate – $MgSo_4$. $7H_2O$	1,150.0
Magnesium Chloride – $MgCl_2$. $6H_2O$	900.0
Calcium Chloride – $CaCl_2$. H_2O	233.6
Potassium Chloride – KCl	100.0
Sodium Bicarbonate – $NaHCO_3$	33.5
Potassium Bromide – KBr	4.5

Monitoring of fish mortality in the various treatments was done every three hours until 96 h period. Dead fish were removed immediately using a scoop net after showing no response to external stimuli. Moribund specimens were also declared as dead samples. Water quality parameters such as dissolved oxygen (mg L^{-1}) and temperature (°C) were measured using a dissolved oxygen meter (Yellow Spring Instruments Model 51B Simpson Electronic Co., IL). The level of pH was determined using a handheld pH meter

(Oakton pH tester 30). Such variables were monitored twice a day at 0900 and 1500 h. Total Ammonia Nitrogen (mg L^{-1}) was measured once daily using an ammonia test kit (API Test kit).

Data analyses. The LC_{50} was determined using two ways: a) a graphical method by intersecting the 50% survival rate and 50% mortality rate; and b) the use of Probit analysis wherein the lethal concentration was based on non-overlapping confidence intervals at 95% (Bringolf et al 2005). Data on the survival of *L. plumbeus* exposed to various levels of salinity were analyzed using the log-rank Kaplan-Meier (KM) function. All analyses were done using Paleontological Statistics v 3.0 and SPSS v 15.0 for Windows.

Results

Survival rate. The cumulative dataset of surviving fish samples in the experiment is presented in Table 2. Initial evaluation of survival data revealed the capacity of *L. plumbeus* to tolerate low level of salinities as zero mortality was observed in treatments 0 and 7.5 g L^{-1} until 96 h. Mortalities were observed in treatments 15, 20, and 25 g L^{-1}, with ascending dosage-time response and increasing salinity, albeit the mortality rate did not exceed 7% in each treatment. Further, acute toxicity test revealed survival rates of 48.89% and 37.78% at 24 h exposure time in treatments 30 g L^{-1}, and 35 g L^{-1}, respectively. The overall survival rate in 35 g L^{-1} was 31.11%, which occurred within the 48-h exposure time. Log-rank analysis of KM survival function revealed significant difference (X^2 = 123.76; df = 6; $p < 0.01$) of sample survival times in different salinity concentrations.

Table 2

Data on the survival (%) of juvenile *L. plumbeus* during the salinity toxicity testing in a direct transfer exposure experiment

Salinity (g L^{-1})	*Survival (%)*			
	24 h	*48 h*	*72 h*	*96 h*
0	100	100	100	100
7.5	100	100	100	100
15	100	100	100	97.78
20	100	100	95.56	95.56
25	100	93.33	93.33	93.33
30	48.89	48.89	48.89	48.89
35	37.78	31.11	31.11	31.11

Median lethal concentration of salinity. The graphical method of determining LC_{50} of salinity on *L. plumbeus* is depicted in Figure 1. The median lethal concentration of salinity using this method indicates that toxicity started around 29.92 g L^{-1}. It is apparent that several *L. plumbeus* specimens were able to survive even at high salinity levels (30-35 g L^{-1}) until 96-h exposure time (Table 2 and Figure 1). On the other hand, Table 3 shows the LC_{50} and confidence limits computed using Probit analysis. The estimated LC_{50} of salinity was 30.70 g L^{-1}, with confidence limits of 28.20-34.24 g L^{-1}. The LC_{50} showed a significant difference due to non-overlapping salinity values in contrast to the other LC points that exhibit overlaps. The Probit analysis estimated that half of the population showed sensitivity to salinity concentration of 30.70 g $L^{-}1$, which can lead to mortality. It is highly observable that mortality increases with increasing salinity levels. For the whole duration of the experiment, no mortality in the test fish was observed in treatments 0 and 7.5 g L^{-1}.

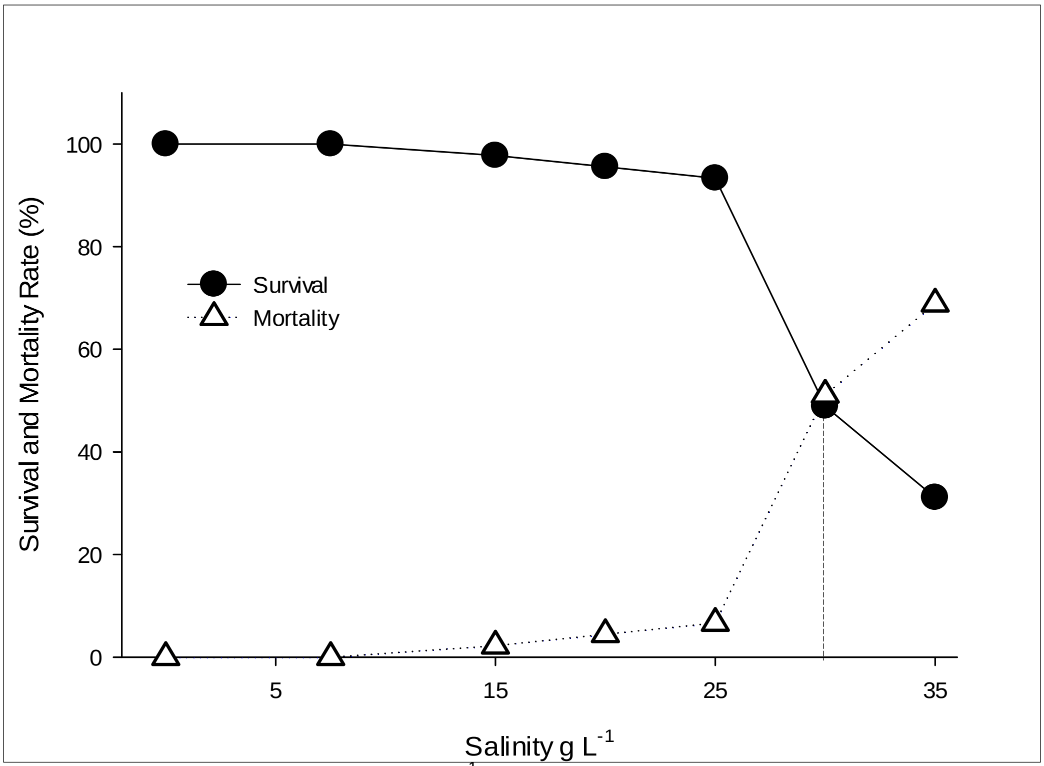

Figure 1. The LC_{50} in salinity of 29.92 g L^{-1} of *L. plumbeus* based on the graphical method by intersecting the survival and mortality rates. The dashed line indicates the estimated concentration.

Table 3

Acute salinity toxicity of *L. plumbeus* after 96-h test. Concentration and confidence intervals are estimated using Probit analysis

Lethal concentration (%)	*Concentration (g L^{-1})*	*95% confidence limits for salinity*	
		Lower bound	*Upper bound*
1	16.07	5.09	20.54
5	20.36	12.69	23.72
10	22.64	16.64	25.52
15	24.19	19.22	26.82
20	25.41	21.19	27.93
50	30.70	28.20	34.24
85	37.22	33.82	45.02
90	38.76	34.98	47.74
95	41.05	36.66	51.80
99	45.33	39.74	59.51

***Estimated time of* survival**. Table 4 shows the results of the time-to-event model with the test terminated at 96 h. The log-rank KM function revealed that *L. plumbeus* was able to tolerate low salinities (0-25 g L^{-1}). At 25 g L^{-1}, samples are assessed to survive up to nearly four days (90.80 h), whereas at 30 g L^{-1}, specimens were found to survive up to almost two days (47.20 h); specimens in full-strength seawater (35 g L^{-1}) had survived until one and a half-day.

Table 4
Estimated time (h) of survival of *L. plumbeus* under varying concentrations of salinity with 96 h as the endpoint

Salinity	*Mean*				*Median*			
	Estimate	*SE*	*95% CI*		*Estimate*	*SE*	*95% CI*	
			Lower	*Upper*			*Lower*	*Upper*
0	96.00	0.0	96.00	96.00				
7.5	96.00	0.0	96.00	96.00				
15	94.73	1.25	92.28	97.19				
20	93.73	1.57	90.66	96.81				
25	90.80	2.90	85.11	96.49				
30	47.20	5.96	35.52	58.88	21.00	2.47	16.17	25.83
35	36.40	5.80	25.04	47.77	12.00	1.43	9.20	14.80
Overall	72.57	2.48	67.72	77.43				

SE = standard error; CI = confidence interval.

Water quality. Water quality parameters including temperature (24.06±0.05°C) and pH (7.59±0.11) were within the tolerable level for the growth and survival of tropical fishes (Boyd 1998). The dissolved oxygen level however was fluctuating between 2.1 and 3.5 mg L^{-1}, which is below the ideal 5 mg L^{-1}. Total ammonia nitrogen was recorded to be high (1.12±0.12 mg L^{-1}).

Discussion

Acute salinity tolerance. This preliminary study demonstrated that *L. plumbeus* can tolerate a wide range of salinity and have a strong chance of survival even in average seawater salinity. The salinity tolerance (acute LC_{50}) of *L. plumbeus* exposed to direct transfer method is relatively similar to congeneric *L. unicolor* juveniles (32.4 g L^{-1}), but higher than in silver perch, *Bidyanus bidyanus* juveniles (20.1 g L^{-1}) (Allen 1982 as reported by Dunlop et al 2005). In Eurasian perch (*Perca fluviatilis*), LC_{50} was attained in 13.0 g L^{-1} and 18 g L^{-1} at 62-h and 39-h exposure time, respectively (Overton et al 2008), whilst 11.8 g L^{-1} was recorded for Sooty grunter, *Hephaestus fuliginosus* (Bisson & Bartholomew 1984). Apart from the abovementioned terapontids, the present result is also comparable to the reported survival capacity of several euryhaline teleosts when challenged to different salinity levels (Ahokas & Duerr 1975; Whitfield et al 1981; Al-Amoudi 1987; Lisboa et al 2015). Decreasing survival rates of freshwater fishes with increasing salinity have been extensively discussed as a result of osmoregulatory stress due to increasing osmotic maintenance requirements at higher salinities (McDonald & Milligan 1997; Kültz 2015).

It is noteworthy to mention the observed zero mortality of *L. plumbeus* at 0 to 7.5 g L^{-1}, and very high survival percentage (≥ 94 %) in salinities up to 25 g L^{-1}. In comparison, the closely related species, *B. bidyanus* was reported to tolerate 12 ppt salinity without mortality, but its survival gradually decreased with increasing salinity (Guo et al 1995). In the present study, when *L. plumbeus* was exposed to higher salinities (30-35 g L^{-1}) heavy mortality was observed, however, this observation only occurred during the first 24 h exposure, and prolonged exposure (72 to 96 h) to high salinities only resulted in minimal or zero mortality (Table 2). This particular result opens the possibility that the survival of *L. plumbeus* could be much higher if introduced in water with gradual changes in salinity rather than abrupt changes, as modeled in the direct transfer toxicity test employed in the present study. In several laboratory studies, the slow increase of salinity over time increases the chances of survival of freshwater fish (chronic LC_{50}) as compared with direct transfer studies (acute LC_{50}) (see Dunlop et al 2005). In a natural setting, salinity fluctuation would be gradual, and this would likely allow several fish species to tolerate higher salinity than in direct transfer (Bringolf et al 2005).

The present study focused on mortality as the endpoint, similar to what has been done by other authors (Bringolf et al 2005; Brion et al 2013). The toxicological relevant effects (e.g., local irritation of gill tissues, skin lesions, etc.) of salinity are therefore not evaluated. The specimens that survived in the experiment may have attained histological and physiological damages during or after the experiment. In-depth evaluation of these negative impacts of salinity on *L. plumbeus* is important and this is open for future investigations.

Conclusions. This preliminary study supports the view that *L. plumbeus* is not exclusively restricted to freshwater habitat as specified in FishBase. Based on available literature, and this study, evidence suggests that *L. plumbeus* can be categorized as secondary freshwater fish with a potential to tolerate a marine salinity. Information on salinity tolerance of *L. plumbeus* could be useful in defining its dispersal and migratory capability, especially in the coastal or downstream fluvial region.

The results of the study could also provide vital information on the domestication of *L. plumbeus* for the future brackishwater aquaculture. As the demand for freshwater is continuously increasing due to agricultural, industrial, and domestic purposes, utilization of brackishwater as a potential culture medium for *L. plumbeus* is an advantageous strategy. Understanding the LC_{50} could aid in the selection of a particular population or stock that is well-adapted to oscillating saline conditions. Nevertheless, further researches are essential to shed light on the possibility of brackishwater silver therapon production.

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