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CHANGES IN SUBLETHAL EFFECTS AND LEAD ACCUMULATION IN *ACANTHOPAGRUS LATUS* UNDER VARIOUS LEAD CONCENTRATIONS AND SALINITIES

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Key words: *Acanthopagrus latus*, lead, salinity, lethal effect.

ABSTRACT

This study investigated the sublethal concentration of lead and calcium levels in yellowfin seabream juvenile (*Acanthopagrus latus*) (4.367 ± 0.470 cm; 1.408 ± 0.478 g) following exposure to a series of lead concentrations at various salinities (0‰, 9‰, 17‰, 25‰ and 34‰).

Yellowfin seabream juveniles exposed to 17‰ salinity were the most tolerant to lead. After 96-h, the 50% lethal concentration (LC_{50}) values were 0.33, 1.43, 3.78, 2.32 and 1.46 mg Pb^{2+}/L following exposure to 0‰, 9‰, 17‰, 25‰ and 34‰ salinities, respectively. The 6-, 12-, 24-, 48- and 96-h LC_{50} for fish exposed to 17‰ salinity were 124.67, 33.02, 10.40, 5.15 and 3.78 mg Pb^{2+}/L, respectively.

The lead levels in fish body increased with increasing ambient lead concentrations in each acclimated salinity. Fish juvenile exposed to 17‰ salinity had the lowest lead accumulation compared with other exposure salinity exposures. The calcium levels decreased with decreasing salinity exposure and increasing ambient lead concentrations. The fish juvenile exposed to low salinity had a Pb^{2+}/Ca^{2+} ratio that was significantly higher than the high salinity experimental group with the same lead concentration exposure. In conclusion, the lead toxicity and accumulation of the fish juvenile were affected by salinity concentrations.

I. INTRODUCTION

Heavy metals are major contaminants that pose a threat to human health and aquatic lives globally (Al-Yousuf et al., 2000; Edwards et al., 2001). Most human activities cause environmental pollution, with pollutants directly or indirectly entering rivers, lakes, and the marine environment (Otitoloju, 2003). Lead is a group-IV metal element in the Periodic Table. It has been mined and used by industries and in household products for centuries. Currently, the most common occupational and environmental routes of exposure to lead are through manufacturing lead batteries, glass, paints, rubber products, and other articles for daily use. For these multiple purposes, lead is a ubiquitous heavy metal. Lead is persistent in water and soil environments, and can be found in most environments. Compared with other pollutants, lead will remain in the environment for a long time, therefore, lead and its compounds easily remain in the soil and mud. Lead can also easily enter the food chain and affect organisms. Over 4 million tons of lead are mined each year and the existing environmental lead levels are at least 500-times greater than prehistoric levels. (Jeng et al., 2000; Hung et al., 2001; Shulkin et al., 2003). Lead has various form freshwater and sea water. The average concentration is 3.9 ug/L in surface water and 0.005 ug/L in sea water. But river sediments contain about 20 mg/g and coastal sediments about 100 mg/g. (EPA, 1989; ATSDR, 1993). The lead and its compounds levels in soil or river sediment to sea via the river. For example, the lead levels were 9.5-470 mg/kg in sediment of Kaohsiung Harbor, Taiwan (Chen et al., 2007).

Like other heavy metals, lead readily accumulates in the ambient environment and can reach highly toxic levels of aquatic life. The most common forms in natural bodies of water are $Pb(CO_3)_2$, Pb^{2+}, and $Pb(OH)^+$; and, Pb^{2+} and $Pb(OH)^+$ are more toxic than the other forms (Hodson et al., 1978). Lead reacts with organic compounds and becomes organic lead, such as tetramethyl lead ($Pb(CH_3)_4$), tetraethyl lead ($Pb(C_2H_5)_4$), and lead acetate ($Pb(CH_3COO)_2$). Arcega-Cabrera et al. (2010) indicated that organic lead compounds, such as tetramethyl lead and tetraethyl lead, are more toxic than other organic and inorganic lead compounds.

Most of the lead in environmental media are from anthropogenic sources, and it has different fates in fresh water compared with seawater. Contents of lead and its compounds enter

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from rivers into the soil and sediments of the sea. Therefore, marine live in estuaries and coastal areas may experience intense pollution (Huang and Onyx, 2004). Heavy-metal pollution is a global issue. Most heavy metals enter marine ecosystems via wastewater from human activities (Kotnik et al., 2000; MacKenzie and Pulford, 2002; Ettler et al., 2004, 2005). Heavy metals pollute water and sediments, causing toxic outcomes for aquatic organisms (Bryan, 1979; Hall et al., 1995).

Yellowfin seabream (*Acanthopagrus latus*) is a favorite species cultured and caught wild in Asian countries. *A. latus* distributes widely in coastal waters and estuarine zones throughout the Indo-West Pacific Ocean (Carpenter, 2001; Hesp et al., 2004). It is also an important and sizable economic fish for aquaculture in Taiwan (Liao et al., 2001). Meanwhile, *A. latus* can adapt to a variety of salinity conditions (Kasahara, 1957). Coastal areas and estuaries are important habitats for *A. latus*, and unfortunately, these areas are also the most polluted due to human activities and salinity changes from heavy rainfall. Therefore, this study examined how various salinities and lead levels affect the lethality of yellowfin seabream.

II. MATERIALS AND METHODS

The artificial seawater used in this study was modified from Wang et al. (2012), and composed of 29 g/L NaCl, 0.7 g/L $CaCl_2 \cdot 2H_2O$, 0.8 g/L KCl, 2.54 g/L $MgCl_2 \cdot 6H_2O$, and 3.415 g/L $MgSO_4 \cdot 7H_2O$ and based on crude salt. The salts were completely dissolved after one day; then municipal tap water was adjusted to 0‰, 9‰, 17‰, 25‰ and 34‰ salinities, and filtered through a gravel-and-sand bed by air-lifting and aeration for three days before use. The lead test solution was prepared by dissolving $Pb(NO_3)_2$ in 1 L of distilled water to make a 1000 mg/L stock solution which was then diluted to a series of desired concentrations (0, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 80, 100, 120 and 150 mg/l) in the prepared artificial seawater at various salinities. The lead and calcium concentrations of the test solutions were measured on a flame atomic absorption spectrometer (Spectra A-240FS, Varian, Palto Alto, CA, USA).

Yellowfin seabream juvenile (*Acanthopagrus latus*) (4.367 ± 0.470 cm; 1.408 ± 0.478 g) were obtained from the aquatic animal center at the National Taiwan Ocean University and acclimated in fiberglass-reinforced plastic tanks at a salinity of 34‰ for two weeks prior to experimentation. Fish were randomly divided into five groups at the same salinity, and then adjusted by 2‰~3‰ each day until the salinity levels of 0‰, 9‰, 17‰ and 25‰ were reached. After the fish had reached the expected salinity levels, they were reared for one more week. Fish were fed twice daily with commercial fish food specifically formulated for mullet fry (with 43% crude protein) manufactured by Fuyu Products (Taichung, Taiwan) during the acclimation and experimental periods. The mean (± SD) body length and wet body weight did not significantly differ among treatments.

Short-term 50% lethal concentration (LC_{50}; median lethal concentration) toxicity tests were carried out in triplicate according to the methods of Cheng and Chen (1994). Toxicity experiments to establish tolerance limits were conducted in 25-L polyethylene tanks containing 20 L of the test solution using the semi-static renewal method (Buikema et al., 1982). The tank was aerated by an air blower with an aeration stone and covered with a plastic cap to prevent the fry from escaping. Each tank contained 30 fish juveniles. Observations were made every 30 min to record the survival up to 96 h. A fish was assumed to be dead when it was immobile and showed no response. The body length and weight of each dead fish was measured.

The water temperature was maintained at 25 ± 1°C and dissolved oxygen (DO) at 7.83 ± 1.62 mg/L, and pH values were 8.08 ± 0.02, 8.12 ± 0.03, 8.19 ± 0.04, 8.28 ± 0.03 and 8.40 ± 0.03 at the respective salinities of 0‰, 9‰, 17‰, 25‰ and 34‰.

The LC_{50} values of lead and their 95% confidence limits were analyzed with a computer probit program. The estimated probit line and results of a χ^2 (Chi-squared) test for the goodness of fit were computed with the probit program. The calculated LC_{50} values were subjected to one-way and two-way analyses of variance (Steel and Torrie, 1980). If significant differences were indicated for the $p < 0.05$ level, then Duncan's multiple-range test was used to identify significant differences between treatments with SAS (Statistical Analysis System, Cary, NC, USA) vers. 6.03. All tests were significant at the $p < 0.05$ level.

All the fish were sampled after 96-h, then the fish fry exposed to the 0, 0.2, 0.5, 1, 2 and 5 mg Pb^{2+}/L environments were removed immediately and frozen at -20°C. Then, samples were dried out with a freeze-dryer. Concentrated nitric acid (70%) was added to samples and digested in a microwave-accelerated reactor (MARS Xpress, CEM, Matthews, NC, USA), and the whole body Pb^{2+} and Ca^{2+} ions were determined using a flame atomic absorption spectrophotometer (SpeactAA 240-FS, VARIAN, Palo Alto, CA, USA). The ratio of Pb^{2+}/Ca^{2+} was calculated for the value of Pb^{2+}/Ca^{2+}.

The body Pb^{2+} and Ca^{2+} ion concentrations were subjected to one- and two-way analyses of variance (Steel and Torrie, 1980). If significant differences were indicated at the $p < 0.05$ level, then Duncan's multiple-range test was used to identify significant differences between treatments. Statistical significance of all tests was accepted at the $p < 0.05$ level.

III. RESULTS

At the same experimental time period, fish acclimated to 17‰ salinity had the highest LC_{50} value, and there were significant differences ($P < 0.05$) between fish acclimated to 0‰ 9‰, 17‰, 25‰ and 34‰ salinity. The environment with 0‰ salinity, had LC_{50} values of 4.18, 1.10, 0.60, 0.43 and 0.33 mg/L at 6, 12, 24, 48, and 96 h, respectively. The environment with 9‰ salinity, had LC_{50} values of 22.90, 7.51, 3.44, 2.14 and 1.43 mg/L at 6, 12, 24, 48 and 96 h, respectively. The environment with 17‰ salinity, had LC_{50} values of 124.67, 33.02, 10.40, 5.15 and 3.78 mg/L at 6, 12, 24, 48, and 96 h, respectively. The environment with 25‰ salinity, had LC_{50} values were 96.88, 18.93, 3.77, 2.80 and 2.32 mg/L at 6, 12, 24,

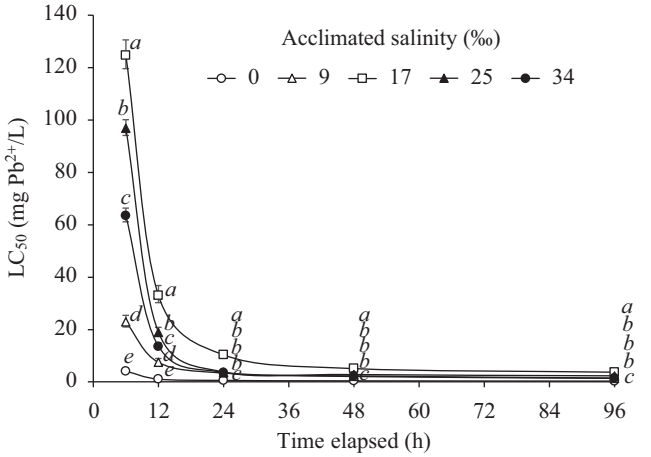

Fig. 1. Lead 50% lethal concentration (LC_{50}) values (mg/L) and their 95% confidence limits for *Acanthopagrus latus* at various salinities. Data at the same exposure time with different letters are significantly different among the various ambient salinities ($p < 0.05$).

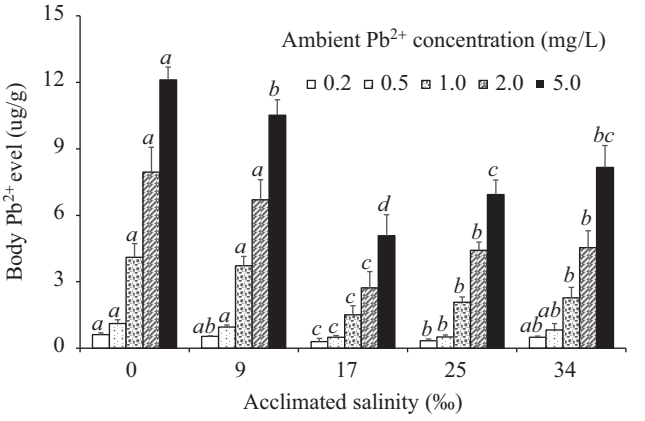

Fig. 2. Body Pb^{2+} levels (μg/g) in *Acanthopagrus latus* acclimated to 0‰, 9‰, 17‰, 25‰ and 34‰ salinity and exposed to 0.2, 0.5, 1, 2 and 5 mg/L lead concentration for 96 h, respectively. Data at the same acclimated salinity with different letters are significantly different among the various ambient lead concentrations ($p < 0.05$).

48, and 96 h, respectively. The environment with 34‰ salinity, had LC_{50} values of 63.47, 13.59, 3.65, 2.27 and 1.46 mg/L at 6, 12, 24, 48, and 96 h, respectively (Fig. 1).

The body Pb^{2+} levels in the controls (without added Pb^{2+}) were not detected (limited concentration was 10 ng/L). The body Pb^{2+} levels increased with increasing ambient lead concentrations. The highest Pb^{2+} level occurred in the fish exposed to 0‰ salinity for each ambient Pb^{2+} concentrations. Then, Pb^{2+} levels decreased with increasing ambient Pb^{2+} concentrations within fish acclimated to 0-17‰ salinity. In the fish juvenile acclimated to 17-34‰ salinity, body Pb^{2+} levels increased with increasing exposure to salinity in the same ambient Pb^{2+} environments. Fish exposed to an environment with 0‰ salinity had Pb^{2+} levels of 0.621, 1.114, 4.110, 7.948 and 12.106 μg/g wet weight compared with fish acclimated to 0.2, 0.5, 1, 2 and 5 mg Pb^{2+}/L, respectively. Fish exposed to an environment with 17‰ salinity had body Pb^{2+} levels of 0.303, 0.495, 1.513, 2.719 and 5.933 μg/g compared with fish acclimated to 0.2, 0.5, 1, 2 and 5 mg Pb^{2+}/l, respectively. Yellowfin seabream juvenile exposed to 0.5 mg Pb^{2+}/L in the environment had body Pb^{2+} levels of 1.114, 0.957, 0.513, 0.495 and 0.823 μg/g following acclimation to 0‰, 9‰, 17‰, 25‰ and 34‰ salinity, respectively. Fish fry exposed to 5 mg Pb^{2+}/L in the environment had body Pb^{2+} levels of 12.106, 10.515, 7.988, 5.933 and 8.164 μg/g following acclimation to 0‰, 9‰, 17‰, 25‰ and 34‰ salinity, respectively (Fig. 2).

The water calcium concentrations were 3.421 ± 0.587, 96.926 ± 1.865, 178.448 ± 5.553, 260.359 ± 8.394 and 349.529 ± 14.883 mg/L in 0‰, 9‰, 17‰, 25‰ and 34‰ salinity, respectively.

Water lead concentrations were not detected in controls. In the other experimental groups, the water lead concentrations were 0.198 ± 0.003, 0.502 ± 0.006, 1.034 ± 0.069, 2.133 ± 0.144 and 5.196 ± 0.440 mg/L for the desired concentrations of 0.2, 0.5, 1, 2 and 5 mg/l, respectively.

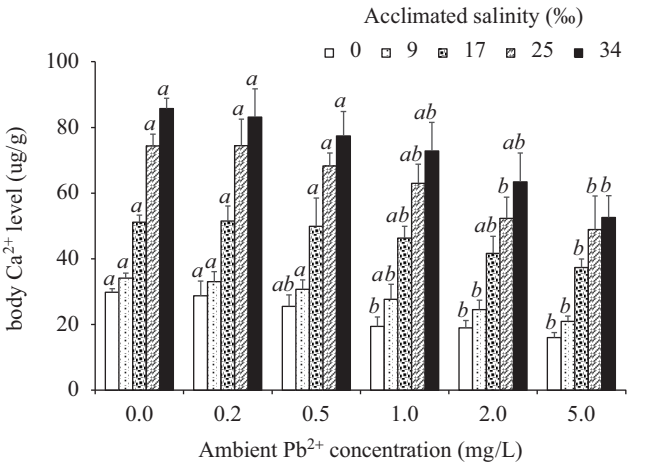

Fig. 3. Body Ca^{2+} levels (μg/g) in *Acanthopagrus latus* exposed to 0.2, 0.5, 1, 2 and 5 mg/L lead concentration and acclimated to various salinities (0‰, 9‰, 17‰, 25‰ and 34‰) for 96 h. Data at the same exposed Pb^{2+} concentrations with different letters are significantly different among the various acclimated salinities ($p < 0.05$).

The fish body Ca^{2+} levels increased with increasing ambient salinity, however decreased with increasing ambient Pb^{2+} concentrations. In the controls (0 mg Pb^{2+}/L), Ca^{2+} levels were 29.864, 34.150, 51.165, 74.356 and 85.787 μg/g wet weight for fish acclimated to 0‰, 9‰, 17‰, 25‰ and 34‰ salinity environments, respectively. The body Ca^{2+} levels were 15.988, 20.952, 37.329, 48.955 and 52.570 μg/g for fish exposed to 5 mg Pb^{2+}/L in the environment and acclimated to 0‰, 9‰, 17‰, 25‰ and 34‰ salinity, respectively (Fig. 3).

Fish exposed to the same ambient Pb^{2+} environment had Pb^{2+}/Ca^{2+} ratios that were significantly decreased with increasing acclimation to 0-17‰ salinity; however, there were no significant changes for 17-34‰ salinity ($p > 0.05$). The Pb^{2+}/Ca^{2+} ratios were 0.006, 0.011, 0.033, 0.065 and 0.136 for fish acclimated to 17‰ salinity and exposed to 0.2, 0.5, 1, 2 and 5

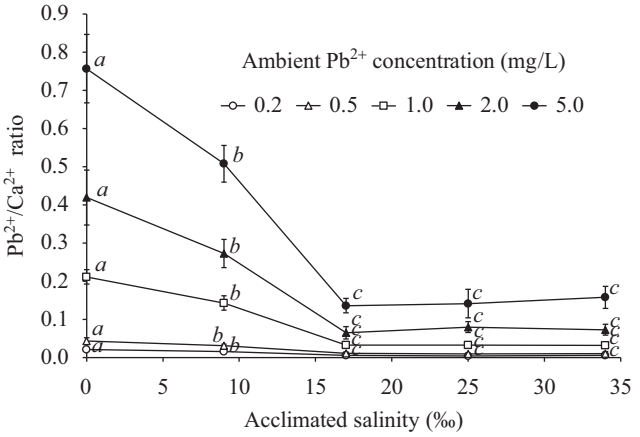

Fig. 4. Pb^{2+}/Ca^{2+} ratio in *Acanthopagrus latus* exposed to 0.2, 0.5, 1, 2 and 5 mg/L lead concentrations for 96 h with salinities of 0‰, 9‰ 17‰, 25‰ and 34‰, respectively. Statistical analysis was previously described in Fig. 2.

mg Pb^{2+}/L in the environment, respectively. The Pb^{2+}/Ca^{2+} ratios were 0.757, 0.508, 0.136, 0.142 and 0.158 for fish exposed to 5 mg Pb^{2+}/L in the environment and salinities of 0‰, 9‰, 17‰, 25‰ and 34‰, respectively (Fig. 4).

IV. DISCUSSION

When $Pb(NO_3)_2$ was added to seawater, a white suspended solid formed. Lead easily forms complexes with OH^- and Cl^- in aquatic systems. This chemical phenomenon causes differences between theoretical and measured lead levels, and was the reason that we used artificial seawater in this study.

Because lead is hard to dissolve in seawater, most of the previous studies used theoretical concentrations to calculate the LC_{50} of lead, therefore it is thus difficult to compare our results with those studies. To compare LC_{50} values of different species, we produced a series of theoretical lead concentrations and measured free Pb^{2+} concentrations to convert theoretical concentrations to measured concentrations for comparison. Lead toxicity differs with the species, size, environment, and exposure time. In general, the LC_{50} decreases with a longer exposure time. In this study, the 48-h LC_{50} values were 0.43, 5.15 and 2.27 mg Pb^{2+}/L at salinities of 0‰, 17‰ and 34‰, respectively. In comparison with the other seawater fish, *Lateolabrax japonicus* and *Micropterus salmoides*, their 48-h LC_{50} values were 83.88 and 37.54 mg Pb^{2+}/L in seawater, respectively (Huang, 1988). The 48-h LC_{50} values for some fish were low, such as *Acanthopagrus schlegli*, *Chanos chanos*, and *A. berda*, with values of 16.10, 15.73, and 13.62 mg/L, respectively (Chen and Ting, 1990; Wai et al., 1984). In comparison to other freshwater fish, respective 48-h LC_{50} values of *Misgurnus anguillicaudatus*, *Plecoglossus altivelis*, *Cyprinus* sp., and *Oreochromis* sp. were 92.03, 65.77, 2.13, and 1.80 mg/L. At salinities of 0‰ (fresh water) and 34‰ (seawater), lead toxic resistance in *M. cephalus* was lower than those of other marine and freshwater fish. Regardless of whether yellowfin seabream were acclimated to fresh water or seawater, they were highly sensitive to lead toxicity.

We found that the LC_{50} values of yellowfin seabream were significantly different between for fish juvenile acclimated to various salinities in this study. The LC_{50} of fish acclimated to 17‰ salinity was significantly higher than those of fish acclimated to 0‰, 9‰, 25‰ and 34‰ salinity at all exposure times. Other results from our laboratory showed that the LC_{50} values of *Mugil cephalus* at a salinity of 0‰ were significantly lower than those at 17‰ and 34‰ salinity. The LC_{50} of *M. cephalus* acclimated at 17‰ salinity was significantly higher than fish acclimated to 0‰ and 34‰ salinity (Wang et al., 2012). In this study, the acclimated salinity groups were more than Wang et al. (2012). We also found that the LC_{50} for fish acclimated to 17‰ salinity was significantly higher compared with other salinity groups, and the Pb^{2+} levels for fish acclimated to 17‰ salinity were significantly lower than the other salinity groups. Similar results indicated that the lead toxicity of fish was depended on the acclimation to salinity.

The LC_{50} and fish Pb^{2+} levels also presented an opposite trend. LC_{50} values and Pb^{2+} levels of fish acclimated to 17‰ salinity were significantly different than those of fish acclimated to other salinity. Saoud et al. (2007) determined that the isosmotic point of the rabbitfish (*Siganus rivulatus*) was 394.86 mOsm/kg, which was equivalent to 14.85‰ salinity. The isosmotic point of the flatfish (*Dicologoglossa cuneate*) was 10.4‰ salinity (Herrera et al., 2009). Gonzalez-Ortegon et al. (2006) demonstrated that the isosmotic points of shrimp, *Crangon crangon, Melicertus kerathurus, Palaemon serratus, Palaemon longirostris, Palaemon macrodactylus,* and *Palaemonetes varians* were 22.00‰~28.32‰ salinity. However, the isosmotic point of marine teleosts and crustaceans were lower than normal seawater. The toxicity of heavy metals to aquatic animals that live in environments near their isosmotic point may be lower. Wildgust and Jones (1998) noted that opossum shrimp (*Neomysis integer*) mortality was greater at salinities of 28‰ and 12‰ compared with 20‰ salinity. The isosmotic point of *N. integer* is close to 19‰ salinity. This result was similar to the results of this study. In our other experiment, the isosmotic point of yellowfin seabream was 398.6 mOsm/kg, which was equal to 15‰ salinity. Those results may explain why the lowest lead toxicity was found in fish acclimated to 17‰ salinity.

The pathway of lead absorption depends on environmental salinity and osmolality (Somero et al., 1997). There are two types of pathways that aquatic animals use to absorb heavy metals from the environment. The ambient dissolved heavy metal ions accumulate in fish directly through their gill, and fish can absorb heavy metals via food or water in the intestine (Leland and Kuwabara, 1985). Therefore, the gill and intestines are the major organs for fish absorption of environmental lead (Varanasi and Gmur, 1978; Somero et al., 1997). Hamilton and Mehrle (1986) indicated that heavy metal levels of the gill could correlate with environmental heavy metal concentrations directly. Somero et al. (1977) found that the intestine is

the major organ for euryhaline fish to absorb lead and other divalent cations. Seawater fish take in physiological water by drinking water, and expelling surplus water and ions. Therefore, osmolality regulation plays an important role in the mechanism for seawater fish (Belkovskiy et al., 1991). The ambient salinity changes fish osmolality directly. In high salinity environments, fish drink water to regulate osmolality in hypertonic environments. Therefore, fish exposed to 17-34‰ salinity environments have body lead levels that increase with increasing acclimation to salinity.

The toxicity of lead for fish will change depending on the species, size, exposure time and water quality (Health, 1995; Ay et al., 1999). The water calcium ion concentration will change with variations in the ambient salinity. Water calcium ions affect the absorbance of lead. A high calcium level decreases the lead concentration in fish, and also decreases the lead toxicity for fish (Varanasi and Gmur, 1978; Williams and Giesy, 1978). Davies et al. (1976) also indicated that the lead toxicity of rainbow trout (*Salmo gairdneri*) acclimated to soft water (28 mg/L $CaCO_3$) was significantly higher compared with fish acclimated to hard water (353 mg/L $CaCO_3$). Weir and Hine (1970) reported that the LC_{50} for lead in goldfish (*Garassius auratus*) acclimated to 50 mg/L calcium increased from 6.6 to 110 mg/L. In this study, the water calcium ion concentrations were 3.421, 96.926, 178.448, 260.359 and 349.529 mg/l for salinities of 0‰, 9‰, 17‰, 25‰ and 34‰, respectively. Compared with the body lead levels, we found the lead levels increased with decreasing ambient calcium concentrations. Both lead and calcium ion are bivalent cations; therefore, lead ions compete with calcium ion in calcium ion channels.

In this study, we also found that Pb^{2+}/Ca^{2+} ratio had the lowest values with salinities between 17-25‰ and there were no significant differences between salinities of 17-34‰. The body calcium levels were significantly changed with ambient salinity. The high calcium levels resisted lead in the body at high salinities. This result indicated that yellowfin seabreams exposed to different salinities allowed lead to enter the fish via a different pathway.

In conclusion, the lead toxicity of the yellowfin seabream was changed by acclimation to salinity and exposure time. Lead toxicity may be related to acclimating salinity, and the osmolality and isosmotic points of fish.

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