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## Chronic Effects of Lead Exposure on Topsmelt Fish (*Atherinops Affinis*): Influence of Salinity, Organism Age, and Relative Sensitivity to Other Marine Fish

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### Recommended Citation

Reynolds, Erik J.; Hoang, Tham C.; Smith, D. Scott; and Chowdhury, Jasim M.. Chronic Effects of Lead Exposure on Topsmelt Fish (*Atherinops Affinis*): Influence of Salinity, Organism Age, and Relative Sensitivity to Other Marine Fish. *Environmental Toxicology and Chemistry*, , : 26, 2018. Retrieved from Loyola eCommons, Institute of Environmental Sustainability: Faculty Publications and Other Works, <http://dx.doi.org/10.1002/etc.4241>

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*Environ Toxicol Chem.*, **Accepted Article** • DOI: 10.1002/etc.4241

**Accepted Article**

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E.J. Reynolds et al.

Chronic toxicity of Pb to *Atherinops affinis*

**Chronic Effects of Lead Exposure on Topsmelt Fish (*Atherinops Affinis*): Influence of Salinity, Organism Age, and Relative Sensitivity to Other Marine Fish**

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**Submitted 3 May 2018; Returned for Revision 28 May 2018; Accepted 24 July 2018**

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**Abstract:** The aim of this study was to determine the influence of salinity and organism age on the chronic toxicity of waterborne lead (Pb) to *Atherinops affinis* and to compare the relative Pb sensitivity of *A. affinis* with other marine species. Chronic Pb exposure experiments were conducted in a water flow-through testing system. Survival, standard length, dry weight, and tissue Pb concentration were measured and lethal concentrations (LC), effective concentrations (EC), and bioconcentration factor (BCF) were calculated. In general, increasing salinity and organism age decreased Pb toxicity. The LC50s for larval fish at 14 and 28 ppt salinity were 15.1 and 79.8 µg/L dissolved Pb, respectively; whereas, the LC50 for juvenile fish was 167.6 µg/L dissolved Pb at 28 ppt salinity. Using standard length data, the EC10 values for larval fish were 16.4 and 82.4 µg/L dissolved Pb at 14 and 28 ppt salinity, respectively. The dry weight EC25 for low and high salinity were 15.6 and 61.84 µg/L dissolved Pb, respectively. The BCF was higher with the lower salinity study (1,703) in comparison to the higher salinity study (654). Results of Pb speciation calculation showed higher fraction of Pb<sup>2+</sup> in water with lower salinity, explaining the higher observed toxicity of Pb in lower salinity water than higher salinity water.

*Atherinops affinis* is more sensitive to Pb than several other marine species. Evidences for abnormal swimming and skeletal deformities were observed in Pb exposure treatment. Results of the present study are useful for marine Biotic Ligand Modeling and support ecological risk assessment and deriving Pb environmental quality criteria for marine environment. This article is protected by copyright. All rights reserved

**Keywords:** Lead toxicity, growth effect, lead bioaccumulation, bioconcentration factor, lead speciation

## INTRODUCTION

Metal pollution has been an environmental issue for many decades. The introduction of heavy metals to marine ecosystems is concentrated near densely populated coastal areas and industrialized regions. Lead (Pb) has become a metal of concern due to its popular application in a variety of industries. Several metals, such as copper (Cu), zinc (Zn) are both essential to biological function at low concentrations but toxic at high

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concentrations; whereas, Pb is considered a nonessential metal and therefore not required for any known physiological processes.

Lead occurs in the environment as a consequence of both naturogenic and anthropogenic processes.

Activities such as mining, smelting, coal burning, and manufacturing continue to contribute substantial amounts of Pb to aquatic environments (International Lead Association 2017). The world production of lead has increased from 4.602 million tons in 1970 to 11.134 million tons in 2016 (International Lead Association 2017).

Once Pb enters the environment it remains as a persistent contaminant but can form complexes with organic and inorganic ligands which will potentially mitigate its toxicity. Therefore, the increase of Pb production poses potential risks for the environment and chronic toxicity studies are more appropriate to evaluate the environmental risk of Pb.

Most published lead toxicity research has been conducted with acute exposures and freshwater organisms (Pyatt et al. 2002; Grosell et al. 2006; Parametrix 2010; Mager et al. 2010, 2011; Brix et al 2012; Munley et al. 2013; International Lead Association 2017). Literature data for chronic toxicity of lead on marine organisms are limited in part due to the low solubility of lead in marine waters (Taylor et al. 1985; Denton and Jones 1986; Angel et al. 2016; Church et al. 2017). In freshwater, lead primarily exists as the divalent cation ( $Pb^{2+}$ ) under acidic conditions, and forms lead carbonate and hydroxide under alkaline conditions. In seawater, the influence of chloride concentration on dissolved lead speciation is far greater compared to freshwater; the primary dissolved seawater species of lead are chloride and carbonate complexes (Turner et al. 1981). The abundance of dissolved ionic salts in marine environments causes Pb to precipitate out of solution and greatly reduces the bioavailability of Pb (Angel et al. 2016). Angel et al. (2016) present the theoretical limit on lead solubility in seawater as 60  $\mu\text{g/L}$  based on equilibrium with hydrocerussite.

Based on a recent review of literature for the effects of Pb in saltwater by Church et al. (2017), most of the available chronic Pb toxicity data are for marine invertebrate and algal species. In particular, chronic Pb toxicity has so far been reported only for one fish species (*Cyprinodon variegatus*), leading to a poor representation of fish in the species sensitivity distribution used for ecological risk assessment of Pb in marine environment.

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The toxicity of Pb is believed to incorporate both osmoregulatory and ionregulatory stress (Denton and Jones 1986). For metal cations and fish the target site of action, i.e., the biotic ligand, is typically the gills, where metals induce ion regulatory impairments (Taylor et al. 1985). Increased environmental salinity yields fewer interactions between Pb and biotic ligands at the gills due to a cation competition. This competition potentially makes Pb less bioavailable to aquatic organisms and, therefore, would decrease toxicity (Church et al. 2017; DeForest et al. 2017). However, based on a review summary by Church et al. (2017), approximately 72% of the chronic Pb toxicity data were for vertebrate and invertebrate species and at salinity  $\geq 30$  ppt. Studies at salinity between 20 and 30 ppt accounted for only 27%. Further, only 1% of the studies was conducted with brackish water salinity (15%). Given that Pb enters marine environment mainly from human activities on land, coastal environments, typically near river mouths at which salinities are lower than the open ocean, are likely more contaminated with Pb compared to average marine water. Therefore, research on chronic Pb toxicity at salinities representing both inshore and offshore environments are important.

*Atherinops affinis* is a marine fish species that has been used for toxicology research due to its relative sensitivity to a variety of toxicants and its amenability to laboratory culture (Anderson et al. 1991; Middaugh and Anderson 1992; Anderson et al. 1995). The fish is also of high ecological importance as a primary and secondary consumer within the coastal food web (Middaugh and Anderson 1992). Studies show that larval *A. affinis* are amenable to toxicity testing at estuarine salinities from 5 to 33 ppt (Anderson et al. 1995). Although *A. affinis* tolerate lower salinities, they prefer marine conditions. *Atherinops affinis* are abundant in California, Washington, and Oregon along coastlines and within estuaries. The reproductive period of *A. affinis* is during dry summer months at spawn sites such as harbors and estuaries (Anderson et al. 1991). During a spawn migration, *A. affinis* encounter a warm thermocline in the shallow estuary environment and the fish eggs respond to this warmer thermal gradient and hatch (Anderson et al. 1991). It is important to consider the effect of salinity on metal toxicity to species whose choice of habitat is dependent on life cycle and spawn events.

The objective of the present study is to determine the chronic effects of Pb exposure on *A. affinis*; specifically, looking at the influence of salinity and organism age on Pb toxicity to *A. affinis*. Relative sensitivity of *A. affinis* and other marine species to Pb exposure is also discussed in the present study.

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## MATERIALS AND METHODS

### *Experimental design*

Twenty eight day chronic Pb toxicity tests with *A. affinis* were performed in a flow-through test system with synthetic saltwater. Lethal effect concentrations (LCs), effect concentrations (ECs), no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC) were determined based on survival and growth data (fish length and dry weight).

To determine the influence of salinity on chronic Pb toxicity, tests were conducted with  $\leq 3$  d old larval *A. affinis* in 14 ppt and 28 ppt synthetic saltwater. For the influence of organism age on the chronic toxicity of Pb to *A. affinis*, toxicity tests were conducted in 28 ppt synthetic saltwater using  $\leq 3$  d old larval and 2.5 mo old juvenile *A. affinis*. The designed concentrations for test with 14 ppt salinity and  $\leq 3$  d old fish were 0 (control), 25, 50, 100, 150, and 200  $\mu\text{g/L}$  total Pb. For test with 28 ppt salinity and  $\leq 3$  d old fish, the concentrations were 0 (control), 50, 100, 200, 400, and 600  $\mu\text{g/L}$  total Pb. These concentrations were chosen based on the results of a preliminary range finding test conducted in our laboratory (unpublished data). Four replicates per treatment with 10 fish per replicate were designed for these tests. Due to an availability issue with 2.5 mo old fish, three treatments including control (0, 100, 200  $\mu\text{g/L}$  total Pb) were used for test with 28 ppt salinity and 2.5 mo old fish. Two replicates per treatment with 10 fish per replicates were used for this test.

### *Organisms*

Fish were purchased from Aquatic Biosystems Inc. (Fort Collins, Colorado, USA) and priority shipped to the Loyola Ecotoxicology and Risk Assessment Laboratory. Fish were 1 d old and 1 mo old by the time they arrived. Upon arrival, the shipment's water quality parameters (e.g., dissolved oxygen (DO), pH, salinity, conductivity) were checked to ensure the organisms had not undergone physical stress due to low water quality. Fish were then transferred to a holding container, fed freshly hatched brine shrimp (brine shrimp eggs obtained from Brine Shrimp Direct Inc.), and inspected for health. Fish that exhibited abnormal behavior or anatomy were euthanized. The observationally healthy fish (1 d old) were then acclimated to our synthetic saltwater and

laboratory conditions over a period of 24 h but no longer than 2 d prior to testing. Juvenile *A. affinis* (1 mo old) was acclimated to laboratory conditions for 1.5 mo. During acclimation, fish were fed daily with freshly hatched brine shrimp. Once acclimated, chronic Pb toxicity tests were conducted in a water flow-through test system. Synthetic test water was prepared by dissolving an equivalent amount of Crystal Sea Laboratory Bioassay Formula (Marine Enterprises) in 16-18 M $\Omega$  MilliQ water (Barnstead E-pure) to achieve a desired test salinity. To increase solubility of Pb, a small amount of trace metal grade HNO<sub>3</sub> (Fisher Scientific Inc.) was added to the reconstituted seawater (90  $\mu$ L of 50% HNO<sub>3</sub> in 16 L water) to decrease pH to, and maintained at approximately 7.9, during exposure.

### *Toxicity Testing*

Toxicity tests were performed based on the American Society for Testing and Materials 2004 (ASTM 2004) standard guidelines for conducting early life-stage toxicity tests. Test water was synthetic saltwater as described in the organism section. Once prepared, test water was aerated continuously for at least 24 h prior to use for preparing test treatments. Lead stock solution was aliquoted to synthetic saltwater to prepare test treatments. The solutions were then aerated again for at least 24 h for chemical equilibrium before distributing to test chambers. Test chambers were 3 L polypropylene containers. All test chambers were washed with nitric acid, and subsequently rinsed with deionized (DI) water to avoid any interference from metal contamination. A desired amount of Pb(NO<sub>3</sub>)<sub>2</sub> was dissolved in 18M $\Omega$  MilliQ water to produce Pb stock solution of 10,000 mg/L. The solution was subsequently acidified with HNO<sub>3</sub> to a pH of approximately 2 to maximize Pb solubility. Prior to use, the concentration of the Pb stock solution was verified by a NeXion 300S Inductively Coupled Plasma Mass Spectrometer (IC-PMS) (Perkin Elmer Inc.).

All tests were conducted using a water flow-through testing system at a desired temperature of 18 °C and a light photoperiod of 16 h light and 8 h dark in an aquatic toxicology testing room at the Loyola Institute of Environmental Sustainability. Large aerated treatment water basins were placed above the test chamber. A series of peristaltic pumps (Cole Palmer Inc.) aliquoted 83.4 mL to each discrete replicate every 30 min. A hole was bored into each replicate to fit a modified silicone stopper. Once the water level within a replicate exceeded 2 L, treatment water flowed through the modified silicone stopper and exited the test chamber. This ensured



fresh treatment water remained within each replicate volume. The flow-through system was set to replace the total volume of each discrete replicate, 2 L, once every 12 h. The peristaltic pumps were scheduled to turn on every 30 min (Aqua Logic Inc.). All test chambers were placed in a water bed to control the temperature. A water chiller (Pentair Aquatic Eco-Systems, Inc.) was programmed to keep the water bed at 18 °C. Once water in test chambers reaches 18 °C, fish was transferred into test chambers to initiate a test.

Fish were unbiasedly transferred into test chambers one or two at a time to ensure randomization. Fish were only used for testing if they appeared healthy. All tests specimens were fed freshly hatched brine shrimp at least two hours prior to test initiation and daily during the 28 d test period. Dead fish (if observed) was removed from the test chambers daily. At test termination on day 28, surviving fish were collected, rinsed with DI water, euthanized with tricaine methanesulfonate (MS-222) solution, and preserved in 10% formalin solution for later measurement of body weight, standard length, and tissue Pb concentrations. Standard length refers to the length of a fish from the tip of the snout to the last vertebrae and was measured using scientific image analysis software program Image J (SciJava).

To determine fish weight, fish were washed with deionized water to remove any excess saltwater or formalin and weighed on a XS64 Toledo balance with a readability of 0.1 mg to determine wet weight. After wet weight was recorded, the fish were dried in an oven set to 60°C for 24 h to determine dry weight. Fish were then subsequently digested with HNO<sub>3</sub> based on the US EPA Method 3050B for tissue Pb determination (USEPA 1996) and finally analyzed by ICP-MS.

#### *Water chemistry and chemical analyses*

Water quality measurements, such as DO, pH, temperature, and salinity were measured one hour after preparing test solutions and then once a day during subsequent test days. Measurement of pH was performed using a pH meter (model AP110; Fisher Scientific Inc.). Temperature and DO were measured by an YSI 550 meter (YSI Inc.). Salinity was measured by an YSI 30 meter (YSI Inc.). Alkalinity was measured weekly by titrating the test water with 0.02 N H<sub>2</sub>SO<sub>4</sub>.

Water samples were collected at test initiation and weekly throughout the test for total and dissolved metal, anion, cation, and dissolved organic carbon (DOC) analyses. Samples for DOC analysis were collected

into 30 mL amber glass bottles. All other samples were collected into acid washed 15 mL polypropylene tubes.

Dissolved metal, anion, cation and DOC samples were filtered using a 0.45  $\mu\text{m}$  Whatman™ filter (GE Healthcare Life Sciences Inc.). Total and dissolved metal samples were acidified with  $\text{HNO}_3$  to an approximate pH of 2. All samples were stored at 4 °C in a refrigerator prior to analysis. Analysis of metals was performed by ICP-MS. Concentrations of anions and cations were analyzed with an Ion Chromatograph (Metrohm USA Inc.). A TOC analyzer was used to measure concentrations of DOC in the test water (Shimadzu Inc.).

#### *Data Analysis*

Survival data were used to determine LCs. The Probit method was used for calculation of LCs for tests with low and high salinity and larval fish. The LCs for test with high salinity and juvenile fish were calculated using the Linear Interpolation method. Effect concentrations were determined with standard length and weight data and based on Linear Interpolation method. No observed effect concentration and LOEC were determined with both survival and growth data using Dunnett's Test method. All data analyses were performed using CETIS (Tidepool Scientific Software Inc.) and based on the measured dissolved Pb concentrations. CETIS is a statistical package that was developed for analysis of results from environmental toxicity tests. Bioconcentration factor (BCF) for larval fish at low and high salinity was calculated as the ratio of tissue Pb concentration (in dry weight) to the dissolved Pb concentration in the exposure media.

Lead speciation calculations were performed with all stability constants taken as concentration constants as opposed to activity constants. This method was previously reported for copper by Tait et al. (2015). This avoids the need to specify an activity model for seawater (Angel et al. 2016). The specific constants, logK values, for each exposure solution were calculated and the corresponding logK values were interpolated from National Institute of Standards and Technology (NIST 2004) tabulated stability constants over the range 0.0 to 1.0 mol/L ionic strength. The ionic strength of the 28 ppt exposure water were assumed to be 0.61 mol/L, and 0.31 mol/L for the 14 ppt exposure water based on 32 ppt seawater having an ionic strength of 0.7 mol/L. Interpolated certified logK values also require total concentrations for each complexing agent. These anion concentrations were determined by measurement and used directly in modelling. For organic matter binding, the conditional logK values of Kozelka and Bruland (1998) were selected corresponding to two binding sites, one

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stronger site at lower concentration (NOM1) and a weaker site at higher concentration (NOM2). The simultaneous equilibrium was presented in tableau notation and solved using an in-house Matlab (Mathworks, Natick, MA, USA) script based on the equations presented in Carayrou et al. (2004). The complete tableaux, including conditional concentration-based equilibrium constants is given as Supplementary Information (Table S11).

## RESULTS

### *Water chemistry and Pb speciation and biological measurement endpoints*

The average measured salinity of test water for the low salinity (14.1 ppt) and high salinity (28 ppt) tests were less than 1% different from the designed salinity (Table 1). The average measured temperature and pH were in the ranges 18.1 to 18.4 °C and 7.92 to 8.02, respectively. Alkalinity of the low salinity water (58 mg/L as CaCO<sub>3</sub>) was approximately half the alkalinity of the high salinity water (105 to 115 mg/L as CaCO<sub>3</sub>) (Table 1). Concentrations of DO were 6.99 to 8.88 mg/L. These DO concentrations are greater than the minimum requirement by the test method (4.5 mg/L). The DOC concentrations of test waters ranged from 1.73 to 2.45 mg/L, values that fall within the normal range of DOC concentrations found in coastal waters (DePalma et al. 2011). Concentrations of anions and cations were within the concentration range of ocean water.

The measured total Pb concentrations for tests with low salinity and larval fish and high salinity and juvenile fish were approximately within 60% of the nominal concentrations (Table 2). Results of QC samples (spike of 1643e, standard reference material of National Institute of Standards and Technology) for Pb showed a recovery within 10% of the certified concentration. Inevitable lead precipitation was visually observed in some of the test waters. As presented in the modeling results, precipitation explains the lower Pb concentrations in the test waters compared to the nominal Pb (Fig. 1A). The measured dissolved Pb concentrations were approximately 30% lower than the measured total Pb concentrations, except for the treatment of 100 µg/L for high salinity and juvenile fish, showing a difference of 50% (Table 2). Lead concentration in the control was below the method detection limit (<0.002 µg/L).

Water chemistry in Table 1 and total Pb concentrations in Table 2 were used to calculate concentrations of free Pb<sup>2+</sup> and other Pb species in the test waters with low and high salinity. At a total Pb concentrations of

approximately  $\leq 25$   $\mu\text{g/L}$ , the total organic Pb (Pb-NOM1 + Pb-NOM2) was the dominant species (Fig. 1A) and accounted for at least 60% of the total Pb in the 14 ppt water (Fig. 1B) or 45% of the total Pb in the 28 ppt water (Fig. 1C). Above 25  $\mu\text{g/L}$  total Pb and for 14 ppt water or between 60 and 170  $\mu\text{g/L}$  total Pb and for 28 ppt water, the total inorganic Pb species became dominant (Fig. 1A). Among the Pb species, free  $\text{Pb}^{2+}$  was one of the least dominant species at both salinity (Fig. 1B, 1C). Free  $\text{Pb}^{2+}$  concentration increased with total Pb concentration and reached saturation at a concentration of approximately 105  $\mu\text{g/L}$  total Pb in the 14 ppt salinity water or 160  $\mu\text{g/L}$  total Pb in the 28 ppt salinity water (Fig. 1A). Salinity affected Pb speciation. At total Pb concentrations greater than approximately 10  $\mu\text{g/L}$ , concentrations of  $\text{Pb}^{2+}$  were higher in the low salinity water than in the high salinity water (Fig. 1A).

### *Toxicity of Pb*

In general, mortality increased with exposure time, especially after about 2 wk of exposure (Fig. S11, Supplemental data). The 28 d total mortality increased and standard length and dry weight decreased with increasing water Pb concentration (Table 2). At high Pb concentrations of tests with larval fish, complete mortality (100%) was observed. Therefore, standard length and dry weight were not determined for these treatments. Mortality of the control ( $\leq 15\%$ ) was less than the acceptable mortality (20%). Tissue Pb concentrations of larval fish ranged from 0.9 (control) to 83.8 mg/kg, dry weight (Table 2).

Salinity strongly influenced Pb toxicity to *A. affinis*. In general, mortality was higher in the lower salinity test than in the higher salinity test (Table 2). As a result, the LC50 (95% CI) value for the 28 ppt salinity test was 79.84 (66.18 - 92.04)  $\mu\text{g/L}$  Pb; whereas, the LC50 (95% CI) value for the 14 ppt study was 15.14 (11.82 - 17.74)  $\mu\text{g/L}$  Pb (Table 3). All other LC values were approximately five times higher for the high salinity test than the low salinity test (Table 3). The NOEC and LOEC were at least four times and six times higher for the high salinity test than the low salinity test (Table 3). These results indicate that Pb toxicity decreased when salinity was increased.

In comparison of the LC values of juvenile fish and larval fish, the LC50 values for juvenile fish (167.60  $\mu\text{g/L}$ ) was approximately 2 times higher than the LC50 value for larval fish (79.84  $\mu\text{g/L}$ ) tested at the same salinity (28 ppt) and 11 times higher than that for larval fish tested at a lower salinity (14 ppt). A similar

difference in other LC50 values for larval and juvenile fish was found (Table 3). These results signify that juvenile fish are less sensitive to Pb than larval fish.

The effect endpoints based on dry weight data also indicate the influence of salinity on Pb toxicity to *A. affinis*. The EC values for test with high salinity were approximately two to eight times higher than the EC values for test with low salinity fish, typically for EC40 (21.47 vs 163.20 µg/L) and EC50 (26.47 vs >171 µg/L) (Table 3). The NOEC and LOEC for the low salinity test were <13.82 and 27.06 µg/L, while they were 45.52 and 89.92 µg/L for the high salinity test, respectively. Similar results were found for standard length data. The EC values for high salinity test was up to 23 times higher than the EC values for low salinity test (Table 3). The BCFs were higher for 14 ppt salinity test than 28 ppt salinity test. On average, the BCF was 1703 for 14 ppt salinity test and 654 for 28 ppt salinity test (Table 3).

## DISCUSSION

### *Influence of salinity on Pb toxicity*

Metal toxicity has been found to be influenced by water chemistry due to complexation with nontoxic components, such as dissolved organic carbon (DOC), carbonate ( $\text{CO}_3^{2-}$ ), chloride, etc. and form various metal species (Mager et al. 2010, 2011; Esbaugh et al. 2011). Among the metals species, free metal or ionic metal is believed to be the most bioavailable and responsible for toxic effect. In the present study, the toxicity of Pb to *A. affinis* was higher in water with low salinity (14 ppt) than in water with high salinity (28 ppt), regardless of the measurement endpoints (Table 3). The results of Pb speciation calculation showed that at the same total Pb concentration, Pb bioavailability ( $\text{Pb}^{2+}$ ) was higher in the 14 ppt salinity water than in the 28 ppt salinity water. This indicates an influence of salinity on Pb bioavailability and would explain the difference in the toxicity of Pb to *A. affinis* at 14 ppt and 28 ppt salinity. The decrease in free ionic  $\text{Pb}^{2+}$  in the high salinity water was likely due to formation of more inorganic soluble lead species, such as chloro complexes (i.e.,  $\text{PbCl}^+$ ,  $\text{PbCl}_2$ ,  $\text{PbCl}_3^-$ ) and  $\text{PbSO}_4$  in the high salinity water compared to the low salinity water (Fig. 1B, 1C, and shown in 1A where the black dashed line is above the blue dashed line).

It is also important to mention that concentration of free ionic  $\text{Pb}^{2+}$  increased with increasing total Pb in the water but was saturated when hydrocerrusite Pb ( $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$ ) begins to precipitate (Angel et al. 2016).

In the present study, the saturation point occurred at a lower total Pb concentration (105 µg/L) in the low salinity water than the high salinity water (160 µg/L) (Fig. 1A). These suggest that Pb bioavailability and toxicity might not significantly change in salinity range of 14 to 28 ppt when total Pb concentrations are greater than 160 µg/L. This could explain the observation of no significant protective effect of salinity on Pb toxicity to mysid *Neomysis integer* at which, the LC (4,274 µg/L Pb) was approximately 27 times higher than the saturation concentration (Verslycke et al. 2003).

The toxicity of Pb is also dependent on the competition with other cations for binding site at the biotic ligand. Free ionic Pb is believed to compete with divalent cations (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) at the biotic ligand (Davies et al. 1976; Mager et al. 2011; Mebane et al. 2012). Lower salinity will increase the total free ionic Pb to cation ratio within the water column because concentrations of cations are lower in lower salinity water than higher salinity water. This will lead to less competition at the biotic ligand, allowing more Pb to be available for uptake and/or toxicity (Blanchard and Grosell 2006; De Polo and Scrimshaw 2012). Lead stresses fundamental biological processes of fish physiology. These interactions occur at the cellular and molecular levels and are a result of the ability of Pb to mimic or displace cations during specific physiologic processes (Baysoy et al. 2013). In the present study, concentrations of divalent cations (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) in the 28 ppt salinity water were approximately 2 to 3 times higher than those in the 14 ppt salinity water. As a result, greater competition between these cations and  $\text{Pb}^{2+}$  at the biotic ligand could likely occur in the 28 ppt salinity water than in the 14 ppt salinity water and resulted in higher Pb toxicity in the lower salinity than higher salinity water in the present study. In addition, behavior and morphological effects were visually observed in the present study. Some fish in Pb exposure treatments but not in the control experienced erratic swimming and skeletal deformities (curved body, Fig. 2). Physical abnormalities, such as black tails, lordoscoliosis (spinal curvature), paralysis, have been observed in rainbow trout chronically exposed to Pb at sublethal concentrations (Davies et al. 1976). Osman et al. (2007) also reported malformation effects of Pb on embryos of the African catfish *Clarias gariepinus* (Burchell, 1822). The physical abnormalities are believed to be associated with direct neurological damage due to lead exposure (Davies et al. 1976). These abnormal swimming and morphological effects would affect fish's

ability to avoid predators and catch its preys - these can lead to population effects. More quantitative study should be conducted to address these consequences of chronic Pb exposure.

At the same salinity, BCF appeared to be lower at higher water Pb concentrations (Table 3). This is in agreement with McGeer et al. (2003) who reported that Pb BCF for fish decreased with increasing water Pb concentration. Results of the BCF support the assumption of competition of Pb and cations at the biotic ligand. On average, the BCF for fish in the 14 ppt salinity water (1,703 L/kg) was approximately 2.6 times higher than the BCF for fish in the 28 ppt salinity water (654 L/kg) (Table 3). These results suggest that higher salinity could cause more cations-Pb competition at the biotic ligand and led to less Pb accumulation in the tissue. The results of Pb accumulation indicate an influence of salinity on Pb accumulation and Pb BCF. These results are in agreement with previous finding on the influence of salinity on Pb accumulation in fish (Somero et al. 1977; Tsui et al. 2016).

#### *Influence of organism age on Pb toxicity*

Based on survival endpoint, results of the present study indicate that larval *A. affinis* are more sensitive to chronic Pb exposure than juvenile *A. affinis*, as the LC values were higher for juvenile fish than for larval fish (Table 3). These results are supported by the general assumption that earlier life stages of fish are more sensitive to metals than later developmental stages due to less development of defense system and detoxification mechanism in younger fish than older fish (Sorensen 1991). The results are in agreement with Marr et al. (1995) who found that larval brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) were more sensitive to metal mixtures (Zn, Cu, Pb, Cd) than juvenile fish. Newly hatched mottled sculpin (*Cottus bairdii*) has been reported to be less resistant to Cd, Cu, and Zn than were older fish (Besser et al. 2007). In addition, Hoang et al. (2004) also reported that 24 h old fathead minnow were more sensitive to Ni than 1 mo old fathead minnow. However, different age/size sensitivities of fish to metals have been reported in the literature. Mebane et al. (2012) found that the sensitivity of rainbow trout to Pb and Zn increased when fish age/size increased from the swim-up fry life stage (approximately 0.1 g) to 0.5 g. For cutthroat trout (*Oncorhynchus clarkii*), a bi-phases sensitivity was observed with the most sensitive to Pb and Zn at the fish size of approximately 0.3 g (Mebane et al. 2012). More research with ages between larval (3 d old) and juvenile

(2.5 mo old) should be conducted to determine whether the bi-phases age sensitivity response to Pb would occur to the *A. affinis*.

#### *Relative sensitivity of A. affinis and other marine species to Pb*

Since salinity and organism age both influenced Pb toxicity to *A. affinis*, to compare the relative Pb sensitivity of *A. affinis* with other species, it was necessary to select literature toxicity data from research conducted under similar salinity, organism age, DOC, and exposure duration to the experimental conditions of the present study. Eight toxicity values (LC10s) ranging from 12.4 to 1,201.7  $\mu\text{g/L}$  were found in the literature (Parametrix 2010; Beiras et al. 2013; Vukov et al. 2013). The salinity for these studies ranged from 25 to 35 ppt. The studies used  $\leq 24$  h old organisms at the start of the exposure and were conducted for 18 d (Beiras et al. 2013), 28 d (Parametrix 2010) or 30 d (Vukov et al. 2013). These experimental conditions are the most similar to the experiment conditions of the present study. These toxicity values were used together with our toxicity value (LC10) for high salinity test and larval fish to construct a species sensitivity distribution (SSD). Results show that *A. affinis* is the second most sensitive to Pb after *A. bahia* and fell in the 20<sup>th</sup> percentile of the distribution (Fig. 3). When incorporating our toxicity value (LC10) into the SSD for chronic exposure to marine fish by Church et al. (2017) without taking the influence of salinity and organism age into account, the distribution percentile for *A. affinis* was about 25%. *Atherinops affinis* was less sensitive to Pb than *Champia parvula*, *Americamysis bahia*, and *Mytilus trossolus* but was more sensitive to Pb than many other species, such as *Cyprinodon variegatus*, *Dunaliella tertiolecta*, *Mytilus galloprovincialis*, *Neanthes arenaceodantata*, *Strongylocentrus purpuratus*. Given the importance of *A. affinis* in the food web of the coastal and marine ecosystems, including *A. affinis* in the SSD for developing environmental water quality criteria is relevant.

#### **CONCLUSIONS AND IMPLICATIONS**

The present study characterized the chronic toxicity of Pb to *A. affinis* and the influence of salinity and organism age on Pb toxicity. Results indicate that Pb toxicity is inversely related to both salinity and organism age. *Atherinops affinis* is relatively sensitive to Pb compared to other marine species. In addition, abnormal swimming and skeletal deformities were observed in the present study. Results of the present study are useful for a potential marine Pb Biotic Ligand Model and support development of species sensitivity distributions for



ecological risk assessment and deriving Pb environmental quality criteria for protection of marine environment, especially for low salinity environments, such as coastal and estuarine ecosystems.

*Supplemental Data*—The Supplemental Data are available on the Wiley Online Library at DOI:

10.1002/etc.xxxx.

*Acknowledgment*—We are grateful to E. Perez, P. Caniff, and C. Mroczkowski for their assistance with conducting the experiments. We acknowledge the financial support of the International Lead Association and assistantship of the Loyola Institute of Environmental Sustainability to this study.

*Data availability*—Data, associated metadata, and calculation tools are available from the corresponding author (thoang@luc.edu).

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## REFERENCES

- Anderson BS, Hunt JW, Piekarski WJ, Phillips BM, Englund MA, Tjeerdema RS, Goetzl JD. 1995. Influence of salinity on copper and azide toxicity to larval topsmelt *Atherinops affinis* (Ayres). *Arch Environ Contam Physiol* 29:366–372.
- Anderson BS, Middaugh DP, Hunt JW, Turpen SI. 1991. Copper toxicity to sperm, embryos and larvae of topsmelt *Atherinops Affinis*, with notes on induced spawning. *Mar Environ Res* 31:17–35.
- Angel BM., Apte SC, Batley GE, Raven MD. 2016. Lead solubility in seawater: an experimental study. *Environ Chem* 13:489–495.
- ASTM International. 2004. ASTM Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. ASTM E124–05. Conshohocken, PA.
- Baysoy E, Gülüzar A, Canli M. 2013. The effects of salinity and salinity metal (chromium and lead) exposure on ATPase activity in the gill and intestine of tilapia *Oreochromis niloticus*." *Arch of Environ Contam Toxicol* 64:2-8.
- Beiras R, González S, Trigo N, Tato T. 2013. Lead full life-cycle toxicity tests with the marine copepod *Tisbe battagliai*. *Australian Journal of Ecotoxicology* 12:73-81.
- Besser JM, Mebane CA, Mount DR, Ivey CD, Kunz JL, Greer EI, May TW, Ingersoll CG. 2007. Relative sensitivity of mottled sculpins (*Cottus bairdi*) and rainbow trout (*Oncorhynchus mykiss*) to toxicity of metals associated with mining activities. *Environ Toxicol Chem* 26:1657-1665.
- Blanchard J, Grosell M. 2006. Copper toxicity across salinities from freshwater to seawater in the euryhaline fish *Fundulus heteroclitus*: is copper an ionoregulatory toxicant in high salinities? *Aquat Toxicol* 80:131–139.
- Brix KV, Esbaugh AJ, Munley KM, Grosell M. 2012. Investigations into the mechanism of lead toxicity to the freshwater pulmonate snail, *Lymnaea stagnalis*. *Aquat Toxicol* 106–107:147–156.
- Carrayrou J, Mose R, Behra P. 2004. New efficient algorithm for solving thermodynamic chemistry. *AIChE* 48:894-904.

- Church BG, Van Sprang, PA, Chowdhury M.J, DeForest DK. 2017. Updated species sensitivity distribution evaluations for acute and chronic lead toxicity to saltwater aquatic life. *Environ Toxicol Chem* 36:2974–2980.
- Davies PH, Goettl Jr. JP, Sinley JR, Smith NF. 1976. Acute and chronic toxicity of lead to rainbow trout (*Salmo gairdneri*) in hard and soft water. *Water Res.* 10:199-206.
- De Polo A, Scrimshaw M. 2012. Challenges for the development of a biotic ligand model predicting copper toxicity in estuaries and seas. *Environ Toxicol Chem* 31:230–238.
- DeForest DK, Santore RA, Ryan AC, Church BG, Chowdhury MJ, Brix KV. 2017. Development of biotic ligand model (BLM)-based freshwater aquatic life criteria for lead following U.S. EPA guidelines. *Environ Toxicol Chem* 36 (11):2965-2973.
- Denton G, Jones C. 1986. Environmental effects on toxicity of heavy metals to two species of tropical marine fish from northern Australia. *Chem Ecol* 2:233–249.
- DePalma SG, Arnold WR, McGeer JC, Dixon DG, Smith DS. 2011. Effects of dissolved organic matter and reduced sulphur on copper bioavailability in coastal marine environments. *Ecotox Environ Safety* 74:230–237.
- Esbaugh AJ, Brix KV, Mager EM, Grosell M. 2011. Multi-linear regression models predict the effects of water chemistry on acute lead toxicity to *Ceriodaphnia dubia* and *Pimephales promelas*. *Comp Biochem Phys C* 154:137–145.
- Grosell M, Gerdes RM, Brix KV. 2006. Chronic toxicity of lead to three freshwater invertebrates-*Brachionus calyciflorus*, *Chironomus tentans*, and *Lymnaea stagnalis*. *Environ Toxicol Chem* 25:97–104.
- Hoang TC, Tomasso JR, Klaine SJ. 2004. Influence of water quality and age on nickel toxicity to fathead minnows (*Pimephales promelas*). *Environ Toxicol Chem* 23:86-92.
- International Lead Association (ILA). 2017. Lead in Aquatic Environments, London, United Kingdom
- Kozelka PB, Bruland KW. 1998. Chemical speciation of dissolved Cu, Zn, Cd and Pb in Narragansett Bay, Rhode Island. *Mar Chem* 60: 267-282.

- Mager EM, Brix KV, Grosell M. 2010. Influence of bicarbonate and humic acid on effects of chronic waterborne lead exposure to the fathead minnow *Pimephales promelas*. *Aquat Toxicol* 96:135–144.
- Mager EM, Esbough AJ, Brix KV, Ryan AC, Grosell M. 2011. Influences of water chemistry on the acute toxicity of lead to *Pimephales promelas* and *Ceriodaphnia dubia*. *Comp Biochem Phys C* 153:82–90.
- Marr JC, Parker AM, Bergman HL. 1995. Relative sensitivity of brown and rainbow trout to pulsed exposures of an acutely lethal mixture of metals typical of the Clark Fork River, Montana." *Can J Fish Aquat Sci* 52: 2005-2015.
- McGeer JC, Brix KV, Skeaff JM, Deforest DK, Brigham SI, Adams WJ, Green A. 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. *Environ Toxicol Chem* 22:1027-1037.
- Mebane CA, Dillon FS, Hennessy DP. 2012. Acute toxicity of cadmium, lead, zinc, and their mixtures to Stream-resident fish and invertebrates. *Environ Toxicol Chem* 31:1334-1348.
- Middaugh DP, Anderson BS. 1992. Laboratory spawning of topsmelt *Atherinops affinis* with notes on culture and growth of larvae. *Environ Toxicol Chem* 11: 393–399. US EPA.
- Munley KM, Brix KV, Panlilio J, DeForest DK, Grosell M. 2013. Growth inhibition in early life-stage tests predicts full life-cycle toxicity effects of lead in the freshwater pulmonate snail, *Lymnaea stagnalis*. *Aquat Toxicol* 128–129:60–66.
- National Institute of Standards and Technology. 2004. NIST critically selected stability constants of metal complexes: version 8.0. NIST Standard Reference Database 46. Gaithersburg, MD.
- Osman AGM, Wuertz S, Mekkawy IA, Exner HJ, Kirschbaum F. 2007. Lead induced malformations in embryos of the African catfish *Clarias gariepinus* (Burchell, 1822). *Environ Toxicol* 22:375-389.
- Parametrix. 2010. Toxicity of lead to the sheepshead minnow *Cyprinodon variegatus*. Corvallis, OR, USA (as cited by Church et al. 2017).
- Pyatt AJ, Pyatt FB, Pentreath VW. 2002. Lead toxicity, locomotion, and feeding in the freshwater snail, *Lymnaea stagnalis* (L.). *Invertebr Neurosci* 4:135–140.

Somero GN, Chow TJ, Yancey PH, Snyder CB. 1977. Lead accumulation rates in tissues of the estuarine teleost fish, *Gillichthys mirabilis*: Salinity and temperature effects. *Arch Environ Contam Toxicol* 6: 337-348.

Sorensen EMB. 1991 Metal Poisoning in Fish. Print. CRC, 1991. Boca Raton, Fl.

Tait NT, Rabson LM, Diamond RL, Cooper CA, McGeer JC, Smith DS. 2015. Determination of cupric ion concentrations in marine waters: an improved procedure and comparison with other speciation methods. *Environ Chem* 13:140–148.

Taylor D, Maddock BG, Mance G. 1985. The acute toxicity of nine grey list metals (arsenic, boron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species: dab (*Limanda limanda*) and grey mullet (*Chelon labrosus*). *Aquat Toxicol* 7:135–144.

Tsui WC, Chen JC, Cheng SY. 2016. Changes in sublethal effects and lead accumulation in *Acanthopagrus latus* under various lead concentrations and salinities. *J Mar Sci Tech* 24:1026-1031. DOI:10.6119/JMST-016-0506-1.

Turner DR., Whitfield M, Dickson AG. 1981. The equilibrium speciation of dissolved components in freshwater and seawater at 25°C and 1 atm pressure. *Geochim Cosmochim Acta* 45:855–881.

US Environmental Protection Agency. 1996. Method 3050B: Acid digestion of sediments, sludges, and soils, revision 2. [July 17, 2018]. Available from: <https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf>

Verslycke T, Vangheluwe M, Heijerick D, De Schamphelaere KAC, Van Sprang P, Janssen CR. 2003. The toxicity of metal mixtures to the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) under changing salinity. *Aquat Toxicol* 64:307–315.

Vukov O, Nasir R, Cunningham J, Smith DS, McGeer J. 2013. Impacts of waterborne Pb to *Americamysis bahia* in a chronic 30 day flow-through exposure. Waterloo, Ontario, Canada: Wilfrid Laurier University.

Figure 1. Lead speciation as a function of total lead (A: blue lines correspond to 14 ppt salinity water and black lines to the 28 ppt salinity water; B: percentage of each speciation in the 14 ppt salinity water; C: percentage of each species in the 28 ppt salinity water).

Figure 2. Morphological effect of Pb on *A. affinis* (photos were taken from two replicates of treatment 1 of the 28 ppt salinity test with juvenile fish; fish with deformities are circled).

Figure 3. Relative sensitivity of *A. affinis* and other marine species to Pb at similar salinity, life stage, and exposure duration. (data for *A. affinis* were from the present study, *C. variegatus* data were from Parametrix 2010, *T. battagliai* data were from Beiras et al. 2013, *A. bahia* data were from Vukov et al. 2013).

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Table 1. Chemistry of test waters (mean  $\pm$  standard deviation)

Test	Salinity (ppt)	Temperature (°C)	pH	Alkalinity (mg/L as CaCO <sub>3</sub> )	DO	DOC	SO <sub>4</sub> <sup>+2</sup>	Cl <sup>-</sup>	Na <sup>+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>
					(mg/L)							
Low salinity and larval fish	14.1 $\pm$ 0.1	18.2 $\pm$ 0.3	7.96 $\pm$ 0.17	58 $\pm$ 5	7.58 $\pm$ 0.39	2.14 $\pm$ 0.53	58 $\pm$ 8	7561 $\pm$ 226	5927 $\pm$ 334	507 $\pm$ 61	138 $\pm$ 24	125 $\pm$ 16
High salinity and larval fish	28.0 $\pm$ 0.6	18.1 $\pm$ 0.2	7.92 $\pm$ 0.07	105 $\pm$ 8	6.88 $\pm$ 0.60	1.73 $\pm$ 0.25	6168 $\pm$ 304	21683 $\pm$ 710	13877 $\pm$ 383	1063 $\pm$ 368	368 $\pm$ 12.0	430 $\pm$ 18
High salinity and juvenile fish	28.0 $\pm$ 0.3	18.4 $\pm$ 0.4	8.02 $\pm$ 0.09	115 $\pm$ 4	8.88 $\pm$ 0.33	2.45 $\pm$ 0.65	3155 $\pm$ 90	15685 $\pm$ 319	10336 $\pm$ 601	1707 $\pm$ 346	591 $\pm$ 132	502 $\pm$ 111

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Table 2. Lead concentrations of test water and measured biological endpoints (mean  $\pm$  standard deviation)

Study	Nominal Pb ( $\mu\text{g/L}$ )	Dissolved Pb ( $\mu\text{g/L}$ )	Total Pb ( $\mu\text{g/L}$ )	Mortality (%)	Standard length (mm)	Dry weight (mg)	Tissue Pb (mg/kg, dw)
Low salinity, larval fish	Control	BDL	BDL	12 $\pm$ 4	14.8 $\pm$ 1.4	4.6 $\pm$ 0.8	0.9 $\pm$ 0.1
	25	14 $\pm$ 1	17 $\pm$ 1	50 $\pm$ 2	13.7 $\pm$ 1.5	3.4 $\pm$ 0.6	27.6 $\pm$ 4.7
	50	27 $\pm$ 2	34 $\pm$ 1	92 $\pm$ 2	12.1 $\pm$ 0.8	2.2 $\pm$ 0.7	38.7 $\pm$ 22.6
	100	51 $\pm$ 3	69 $\pm$ 4	100	N/A	N/A	N/A
	150	80 $\pm$ 7	85 $\pm$ 15	100	N/A	N/A	N/A
	200	117 $\pm$ 19	127 $\pm$ 16	100	N/A	N/A	N/A
High salinity, larval fish	Control	BDL	BDL	13 $\pm$ 11	12.2 $\pm$ 1.3	1.8 $\pm$ 0.2	7.5 $\pm$ 0.6
	50	46 $\pm$ 10	58 $\pm$ 9	18 $\pm$ 9	11.9 $\pm$ 1.5	1.6 $\pm$ 0.2	33.7 $\pm$ 6.9
	100	90 $\pm$ 20	107 $\pm$ 20	72 $\pm$ 6	10.8 $\pm$ 1.3	1.2 $\pm$ 0.0	66.6 $\pm$ 42
	200	171 $\pm$ 22	200 $\pm$ 14	92 $\pm$ 13	9.7 $\pm$ 0.5	0.8 $\pm$ 0.0	83.8 $\pm$ 45
	400	259 $\pm$ 24	386 $\pm$ 43	100	N/A	N/A	N/A
	600	435 $\pm$ 48	563 $\pm$ 45	100	N/A	N/A	N/A
High salinity, juvenile fish	Control	BDL	BDL	15 $\pm$ 7	38.2 $\pm$ 4.0	617.0 $\pm$ 4.1	N/A
	100	100 $\pm$ 21	154 $\pm$ 67	0	34.1 $\pm$ 5.9	532.6 $\pm$ 160.0	N/A
	200	190 $\pm$ 30	239 $\pm$ 98	65 $\pm$ 35	35.6 $\pm$ 2.5	727.5 $\pm$ 88.8	N/A

BDL: below the method detection limit of 0.002  $\mu\text{g/L}$ 

N/A: not applicable



Table 3: Lethal effect concentrations, ECs, NOEC, LOEC, and BCF for *A. affinis* and Pb at different salinities and organism ages ( $\mu\text{g/L}$ )

Endpoints		Low salinity and larval fish	High salinity and larval fish	High salinity and juvenile fish <sup>a</sup>
Survival	LC5	7.68 (3.83 - 10.33)	36.56 (23.68 - 47.39)	105.30 (98.71 - 124.60)
	LC10	8.28 (4.96 - 11.53)	43.44 (29.89 - 54.54)	110.90 (97.11 - 152.40)
	LC15	9.88 (5.90 - 12.45)	48.81 (34.95 - 60.03)	116.80 (95.19 - 183.80)
	LC20	10.70 (6.75 - 13.24)	53.54 (39.53 - 64.84)	123.00 (92.90 - 219.20)
	LC25	11.47 (7.57 - 13.97)	57.96 (43.9 - 69.33)	129.50 (90.23-259.00)
	LC40	13.64 (10.05 - 16.13)	70.79 (56.91 - 82.48)	151.20 (79.58 - N/A)
	LC50	15.14 (11.82 - 17.74)	79.84 (66.18 - 92.04)	167.60 (69.94 - N/A)
	NOEC	<13.82	45.52	N/A
	LOEC	13.82	89.92	N/A
Dry weight	EC5	0.97 (0.18 - 22.78)	1.80 (0.22 - 75.43)	N/A
	EC10	2.90 (0.16 - 23.49)	6.81 (N/A - 81.41)	N/A
	EC15	6.69 (N/A - 23.44)	20.83 (N/A - 97.70)	N/A
	EC20	14.03 (N/A - 21.33)	48.85 (N/A - 129.30)	N/A
	EC25	15.62 (0.36 - 22.74)	61.84 (N/A - N/A)	N/A
	EC40	21.47 (13.87 - N/A)	163.20 (13.90 - N/A)	N/A
	EC50	26.47 (19.16 - N/A)	>171 (N/A - N/A)	N/A
	NOEC	<13.82	45.52	N/A
	LOEC	27.06	89.92	N/A
Standard length	EC5	5.04 (0.076 - 23.76)	56.01 (N/A - 73.72)	N/A
	EC10	16.36 (2.99 - 22.71)	82.43 (51.23 - 116.70)	N/A
	EC15	5.04 (14.98 - 28.46)	117.6 (79.34 - 154.30)	N/A
	EC20	>27 (N/A - N/A)	>171 (N/A - N/A)	N/A
BCF (L/kg, dw)	Treatment 1 <sup>b</sup>	1974	733	N/A
	Treatment 2 <sup>c</sup>	1433	790	N/A
	Treatment 3 <sup>d</sup>	N/A	490	N/A
	Mean $\pm$ Stdev	1703 $\pm$ 383	654 $\pm$ 142	N/A

Data in parentheses for LCs and ECs are 95% confidence intervals

N/A: not applicable

<sup>a</sup>LC values for this test should be interpreted with caution as there were only two treatment concentrations

<sup>b</sup>25  $\mu\text{g/L}$  Pb for low salinity and larval fish test, 50  $\mu\text{g/L}$  Pb for high salinity and larval fish test

<sup>c</sup>50  $\mu\text{g/L}$  Pb for low salinity and larval fish test, 100  $\mu\text{g/L}$  Pb for high salinity and larval fish test

<sup>d</sup>100  $\mu\text{g/L}$  Pb for low salinity and larval fish test, 200  $\mu\text{g/L}$  Pb for high salinity and larval fish test

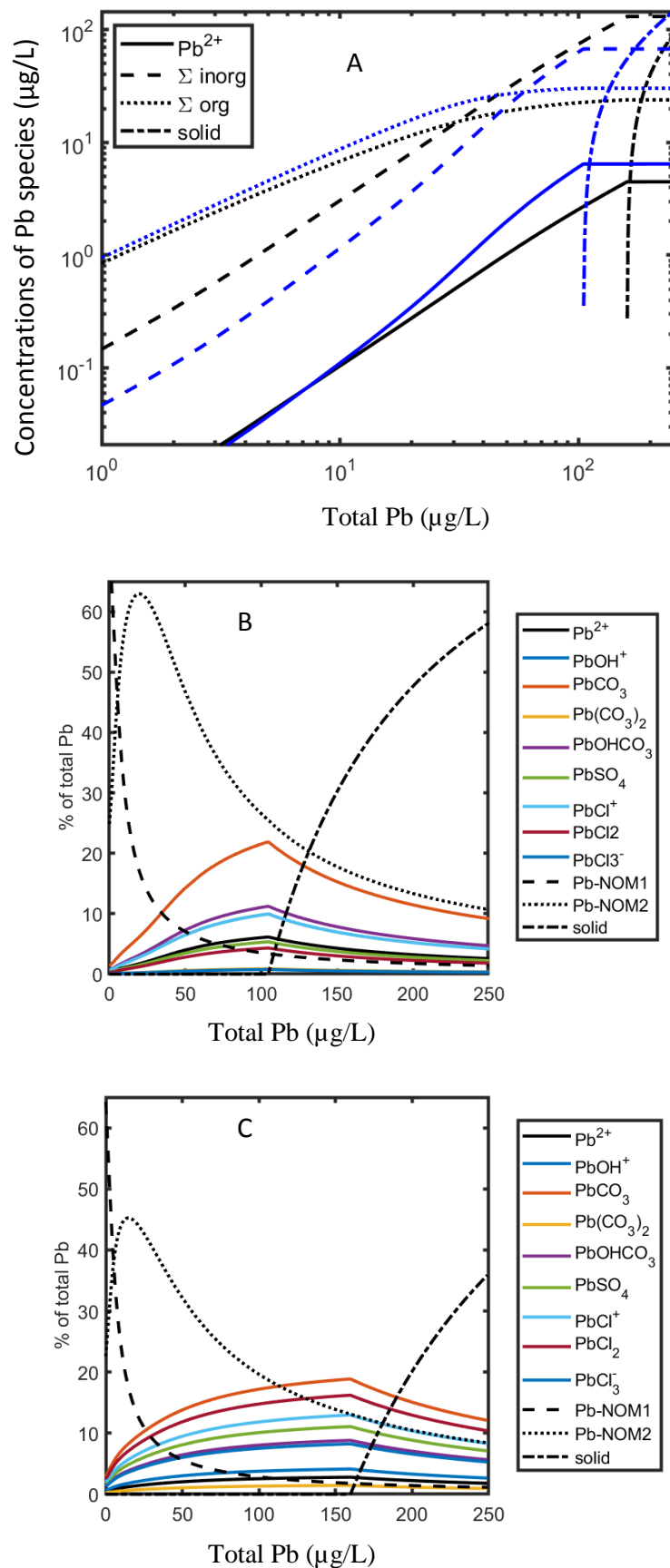


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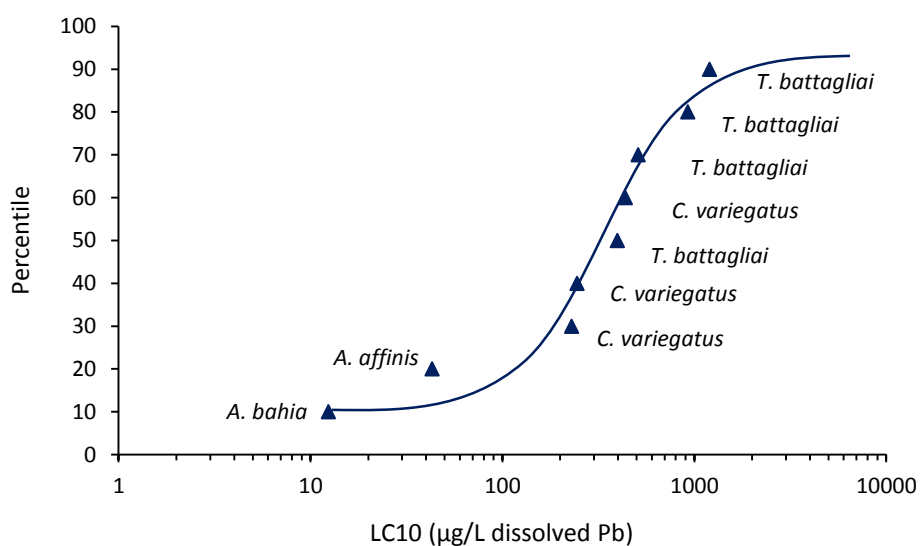


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