Trophic Transfer of Lead Through a Model Marine Four-Level Food Chain: Tetraselmis suecica, Artemia franciscana, Litopenaeus vannamei, and Haemulon scudderi

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BIOLOGICAL RESPONSES OF A SIMULATED MARINE FOOD CHAIN TO LEAD ADDITION

Martín F. Soto-Jiménez,*† Claudia Arellano-Fiore,‡ Ruth Rocha-Velarde,§ Martín E. Jara-Marini,‖ Jorge Ruelas-Inzunza,‡ Domenico Voltolina,# Martín G. Frías-Espericueta,†† Jesús M. Quintero-Alvarez,† and Federico Páez-Osuna†

†Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Mazatlán, Sinaloa, México
‡Technological Institute of Mazatlán, Mazatlán, Sinaloa, México
§Mazatlán Aquarium, Mazatlán, Sinaloa, México
‖Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Sonora, México
#Centro de Investigaciones Biológicas del Noroeste, Mazatlán, Sinaloa, México
††Laboratorio de Estudios Ambientales, Universidad Autónoma de Sinaloa, Mazatlán, Sinaloa, México

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Abstract—This investigation sought to assess the biological responses to Pb along a simplified four-level food chain, from the primary producer, the microalgae *Tetraselmis suecica*, grown in a control medium with < 1 μg/L of Pb and exposed to a sublethal dose (20 μg/L of Pb) and used as the base of a simulated food chain, through the primary-, secondary-, and tertiary-level consumers, namely, the brine shrimp, *Artemia franciscana*; the white-leg shrimp, *Litopenaeus vannamei*; and the grunt fish, *Haemulon scudderi*, respectively. Growth of Pb-exposed *T. suecica* was 40% lower than that of the control cultures, and survival of *A. franciscana* fed this diet was 25 to 30% lower than the control. No differences in the growth rates of Pb-exposed and control shrimp and fish and no gross morphological changes were evident in the exposed specimens. However, the exposed shrimp and fish had 20 and 15% higher mortalities than their controls, respectively. In addition, behavioral alterations were observed in exposed shrimp and fish, including reduction in food consumption or cessation of feeding, breathing air out of the water, reduction of motility, and erratic swimming. The negative correlation between Pb concentration in whole body of shrimp and fish and Fulton's condition factor suggested also that the exposed organisms were stressed because of Pb accumulation.Environ. Toxicol. Chem. 2011;9999:1–7. © 2011 SETAC

Keywords—Lead effects Trophic transfer Bioassay experiment Marine food chain Lead pollution

INTRODUCTION

Lead (Pb) is known to affect growth, development, and reproduction in humans and animals; therefore, Pb contamination of the marine ecosystems has become an environmental problem worldwide, especially in coastal ecosystems, in which it may reach levels of risk for aquatic organisms and for their consumers, including humans [1–3]. The metal content of the diet has been recognized as the main contribution to metal body burden of aquatic organisms and as a major route for transfer through marine food webs [4–7]. Because the metals ingested may become toxic when accumulation in the tissues of aquatic animals reaches a critical level [8], evaluation of the uptake, biotransference, and biomagnification of metals from the low trophic levels to top consumers is critical for determining their effects on marine food chains and their influence on ecosystems and on human life [9–11].

However, the study of transfer processes of metals along food webs and the evaluation of their effects is difficult in the natural environment, so knowledge of their impact on organisms, populations, communities, and ecosystems is still limited. In particular, little information is available on the trophic transfer of Pb in food chains in subtropical ecosystems [12,13], and almost none is available on the effects of the chronic toxicity of Pb for aquatic organisms.

Many bioassays reproducing the natural conditions of an ecosystem have been developed over the past decades, in an effort to understand the relationships between metal transference and its effects on aquatic organisms [13]. However, experimental studies with aquatic food chains exposed to environmentally realistic concentrations of metal through the consumption of whole, living prey organisms are surprisingly rare [5,8,14,15]. Most previous studies have focused on a single species or on no more than two levels of an aquatic food chain and did not use natural chain links, because metals were added by injection into prey items or into ground fish or through direct exposure to waterborne metals in concentrations far in excess of those occurring in most natural waters [14–16].

The present study determined the biological responses to dietary exposure to Pb of organisms of different trophic levels in a simulated marine food chain, chosen in part because of their availability and ease of culture as well as because they are the normal links of some common food chains used in aquaculture, such as microalgae–*Artemia*–shrimp and fish larvae or postlarvae, or because they are links of natural food chains (e.g., shrimp–carnivorous demersal fish).

MATERIALS AND METHODS

The experimental design consisted of feeding the brine shrimp *Artemia franciscana* with the microalgae *Tetraselmis suecica* grown in f/2 medium (control diet) or in the same medium with 20 μg/L Pb. This concentration, which is of the same order of magnitude observed in contaminated coastal environments [17] and between four and five times higher than

* To whom correspondence may be addressed (martin@ola.icmyl.unam.mx).
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concentrations found in Mazatlán Harbor, Sinaloa, México [13], is equivalent to 2.5 times the continuous concentration criterion for Pb (CCC = 8.1 μg/L) ([18]; http://www.epa.gov/ost/criteria/wqctable/). This is an estimate of the level of Pb to which an aquatic community can be exposed indefinitely without an undesired effect and, according to the European Community Dangerous Substances Directive, is considered dangerous for the aquatic environment [19].

The *Artemia* biomass obtained with the control cultures was fed to juveniles of the white-leg shrimp, which in turn served as diet for the carnivorous fish *Haemulon scudderi*. The same food chain, starting with *Artemia* fed with Pb-grown *T. suecica*, served for studying the transference of this metal and assessing its effect through the food chain. Because metal bioaccumulated through dietary exposure can be deposited in different tissues and affect different physiological processes [8,20], we also determined the concentration of Pb in different organs and tissues of shrimp and fish.

All experiments were carried out with natural seawater from Mazatlán Bay, filtered through 2-μm cartridge filters, with an ultraviolet and ozone treatment system. Mean water temperature, salinity, and pH were 28.2 ± 2.0°C, 34.6 ± 1.2 ppt, and 8.1 ± 0.1, and the respective ranges were 28 to 32°C, 34 to 36 ppt, and 7.9 to 8.2. With the exception of microalgae cultures, continuous aeration maintained dissolved oxygen saturation close to 90 to 95% (> 7 mg/L). Light was provided by the natural 14:10 h light:dark photoperiod, and irradiance during daylight ranged from 250 to 1,500 μmol/m^2/s.

Trophic level 1: Microalgae

The control diet was the prasinophyte *T. suecica* (strain TES-1 of the Centro de Investigación Científica y de Educación Superior de Ensenada Culture Collection) [21] grown with the multistep procedure (1, 15, 120 L, with 72 h for each step) in three 160-L fiberglass cylindrical tanks with 120 L of f/2 medium prepared with natural seawater (mean background dissolved Pb concentration 0.29 ± 0.12 μg/L, range 0.18–0.50 μg/L). The experimental diet was grown in three similar tanks with f/2 medium and a suitable volume (depending on the initial Pb concentration) of a 1 mg/L Pb $(NO_3)_2$ stock solution, to obtain a concentration of 20 μg/L Pb.

$Pb(NO_3)_2$ was chosen because Pb(II) is the most stable ionic species and is thought to be the form in which Pb is mostly bioaccumulated by aquatic organisms [22]. This procedure was repeated 10 times to obtain a sufficient amount of *Artemia* biomass for shrimp feeding.

Trophic level 2: Artemia

Nauplii of *A. franciscana* hatched from commercial cysts (INVE Aquaculture) as described by Van Stappen [23] were harvested and transferred to three fiberglass tanks (3,000 L) with approximately 2,500 L of filtered seawater and an initial concentration of 350 to 400 nauplii/L. The Pb-exposed and control microalgae cultures had different cell concentrations. For this reason, daily feeding was provided in two rations of approximately 10 L of the control cultures and approximately 20 L of those exposed to Pb. The resulting concentrations in the *Artemia* cultures ranged between 2.5 and 4×10^6 cells/L. The final harvest was after 19 to 23 d in three (control diet) and six (experimental diet) separate cultures, which were necessary to obtain the amount of biomass for feeding the next links of the control and experimental food chains, respectively.

Trophic level 3: Shrimp

Litopenaeus vannamei juveniles (total wet wt 1.0–1.5 g and total length 5.3–5.7 cm) were obtained from a commercial farm, acclimated during 3 d in three 3,000-L fiberglass tanks with filtered seawater, and fed with nonexposed *Artemia* biomass. After acclimation, groups of 36 specimens were distributed in 16 100-L tanks. The diet of 12 groups, chosen at random, was adult *Artemia* fed with Pb-exposed microalgae. The remaining four received the control diet. In both cases, feeding was ad libitum, twice daily. Excess food was removed after 1 h and, to avoid ingestion of nonexperimental food through cannibalism, molting specimens were removed from the tank.

Trophic level 4: Fish

After a preventive bath [24] (0.025 ml/L formalin and 0.1 mg/L malachite green), in total 120 *H. scudderi* of similar size and weight (7.15 ± 1.2 cm total length and 4.63 ± 1.7 g) captured in Mazatlán Bay were acclimated for 3 d in three 3,000-L fiberglass tanks filled with 5-μm–filtered seawater. After acclimation, groups of 15 fish were separated into each of the eight 300-L tanks and fed daily to satiation with nonexposed shrimp for 6 d. After this period, the fish in five tanks were fed the Pb-exposed shrimp, and the remaining three were fed the control diet.

Determination of sublethal effects

The yields of the microalgae cultures were assessed as cell concentration, determined with a hemocytometer, and growth rates were calculated using the initial and final cell numbers (N). The dry biomass yields were determined using triplicate 20-ml samples, concentrated on precalibrated Whatman GF-C glass fiber filters, rinsed with 3 to 5 ml of a 3% ammonium formate solution, and dried to constant weight at 65°C in a convection oven.

The concentration of the *A. franciscana* cultures was determined at approximately weekly intervals by direct counts of triplicate samples of known volume, from nauplii to the day of the final harvest. In each case, 10 specimens taken at random were measured from the oral to the aboral region under a stereomicroscope with a calibrated eyepiece, and the specimens contained in 1-L sample were concentrated on a 100-μm mesh sieve, counted, and dried as for microalgae to estimate the individual dry weight and the biomass yield.

During the feeding experiments of all trophic levels, dead shrimp and exuviae, as well as dead fish, were removed immediately before adding each of the half-daily food rations. The wet weights and total lengths of five active and apparently healthy specimens of shrimp and fish were obtained weekly, and their individual total weights (W) and lengths (TL) were used to calculate their Fulton's condition factors (K) with the equation $K = 100 \times W/TL^3$. A decrease in this factor may be interpreted as depletion of body reserves, which could be a response to external stressors such as heavy metals [25].

Sampling for Pb analysis

Sample collection and analysis were performed following clean technique protocols [3]. The seawater samples used to determine background Pb concentrations were collected in 1-L polyethylene (LDPE) bottles, previously acid cleaned, filled with 6 N HCl Optimum Grade (Fisherbrand), and stored for at least one month.

The samples (100–300 ml) of *T. suecica* cultures obtained at the time of harvest were concentrated on precombusted (500°C,

4 h), acid-cleaned (2 M HCl) Whatman GF/F glass fiber filters; dried at 55°C to constant weight; and analyzed as for the rest of the samples to obtain the mean Pb contents. Composite samples of *Artemia* (4–5 g wet wt per batch) were obtained with a 200-μm mesh sieve at least 6 h after the addition of microalgae, at approximately weekly intervals and at the end of the growth experiment. By that time, no microalgae were available, *Artemia* had ceased feeding, and their guts were practically empty. For this reason, no further depuration was deemed necessary.

To avoid depuration procedures, the weekly shrimp (four to 12 specimens) and fish (three to five organisms) samples were obtained several hours (6–12 h) after feeding. Shrimp specimens were dissected to separate the muscle, exoskeleton (antennules, carapace, pleopods, and pereiopods), hepatopancreas, and the remaining organs contained in the cephalothorax; in the case of fish, muscle, skeleton (including skull and dorsal and anal fin-rays), skin and scales, gills, liver, viscera, and remaining tissues were separated [13,26]. These samples were frozen and freeze-dried for 72 h. The dried samples were ground, homogenized, and stored in sealed polypropylene containers until analysis.

Analysis

All samples were processed and analyzed in high-efficiency particulate-filtered air (HEPA class 1000) and a trace-metal–clean laboratory using high-purity reagents and water. The powdered samples (100–200 mg) were digested in 30-ml Teflon® vessels with 8 to 10 ml of trace-metal–grade HNO_3:HCl (3:1 v/v), heated overnight at 130°C on a Mod Block unit, transferred to clean polypropylene vials, and diluted to 25 ml with MilliQ water.

The dissolved Pb concentrations of the acidified water and of the biological samples were determined using graphite furnace atomic absorption spectroscopy (GFAAS; Varian SpectrAA 220). Blanks and certified reference material (SLEW-2 estuarine water [$n = 5$], DORM-2 dogfish muscle [$n = 6$], and National Institute of Standards and Technology [NIST] 1566b oyster tissue [$n = 4$]) were analyzed to verify the accuracy of the extraction method. The certified reference material gave the following results: 0.008 ± 0.001 μg/L for SLEW-2, 0.0611 ± 0.012 for DORM-2, and 0.32 ± 0.02 for NIST 1566b (certified values 0.009 ± 0.001 μg/L, 0.065 ± 0.007 μg/g, and 0.31 ± 0.01 μg/g, respectively).

Data processing

The mean cell concentrations, growth rates, dry biomass yields, and Pb contents of the Pb-exposed and control *T. suecica* cultures were compared using Student's *t* tests. The biological responses over time of the Pb-exposed and control cultures of the higher trophic levels were compared using two-way analysis of variance repeated-measures tests, followed by paired *t* tests with the Bonferroni correction. Because the response data collected at different times from the same groups of organisms are not independent, Mauchly's test statistic was used to evaluate whether the relationships between pairs of experimental conditions were similar (assumption of sphericity). The level of significance in all statistical tests was 0.05. Statistica 7.0 (Statistica for Windows; Statsoft) and Office Excel 2007 (Microsoft) were used for all the statistical analyses.

Table 1. Mean cell concentrations (*N*, in 10^6 cells/ml), growth rates (μ in cell divisions/day), dry biomass yields (mg/L), and Pb content (μg/g dry wt) of *Tetraselmis suecica* cultures in f/2 medium (control) or in f/2 medium with 20 μg/L Pb[a]

	Control	Exposed
Cell count (*N*)	0.87 ± 0.05*	0.47 ± 0.06
Growth rates (μ)	1.29 ± 0.11*	1.02 ± 0.16
Biomass	98 ± 10*	48 ± 20
Pb	3.5 ± 1.1*	18.4 ± 3.4

[a] Results are means ± standard deviation; $n = 10$.
* Significant differences (analysis of variance, $\alpha = 0.05$) were observed between control and exposed microalgae.

RESULTS

The mean final cell concentrations, growth rates, and total dry biomass yields of the control cultures of *T. suecica* were significantly higher than those obtained with the Pb-exposed cultures (Table 1). The absorption efficiencies (AE) of these cultures were estimated with the equation $AE = 100$ (Pbi − Pbf)/Pbw, where Pbi and Pbf are the initial and final Pb concentrations of the total microalgal biomass and Pbw is the initial amount of Pb present in the whole volume of the culture medium. The mean Pb content of *T. suecica* grown for 72 h in the Pb-enriched growth medium was 18.4 ± 3.4 μg/g, with an absorption efficiency of 10 to 20% of the added Pb, whereas that of the control cultures averaged 3.5 ± 1.1 μg/g, with an assimilation efficiency $> 70\%$.

Artemia franciscana (level 2)

A tendency to lower survival and lower biomass yields was noted in the exposed cultures, but the differences were significant ($p < 0.05$) only on the day of final harvest (Table 2). A similar tendency existed in the case of total length, but no significant differences were observed throughout the experiment (Table 2). The mean Pb contents of the exposed *A. franciscana* used as food for the next food chain link were 12.0 ± 0.9 to 15.0 ± 1.7 μg/g, which was significantly higher than that of the control *Artemia* cultures (1.3 ± 0.4 to 1.8 ± 0.3 μg/g; Student's *t* tests for paired observations, $\alpha = 0.05$).

Table 2. Mean cell count (ind/L), total length (mm), and dry biomass (mg/L) of *Artemia franciscana* cultures fed *T. suecica* grown in f medium with the background Pb concentration (control) or with 20 μg/L of Pb (exposed)[a]

Day	Cell count (ind/L)		Length (mm; $n = 10$)		Dry biomass (mg/L)	
	Control	Exposed	Control	Exposed	Control	Exposed
1	390 ± 15D	395 ± 17D	0.7 ± 0.1A	0.6 ± 0.2A	12.2 ± 2.8A	11.5 ± 3.0A
7	379 ± 10D	368 ± 14CD	3.5 ± 0.7B	2.9 ± 1.4B	65.0 ± 2.3B	60.0 ± 2.5B
13	353 ± 12C	342 ± 22CB	6.0 ± 0.8C	5.0 ± 1.2C	102 ± 4.0C	94.3 ± 7.6C
19–23	320 ± 20B	255 ± 30A	9.2 ± 1.4D	8.2 ± 1.8D	195 ± 12E	153 ± 18D

[a] Results are means ± standard deviation. Equal letters means no significant differences: A < B < C < D < E.

Litopenaeus vannamei (level 3)

A general tendency toward lower survival and total length and weight was observed in the Pb-exposed shrimp cultures, but the differences were significant only on the final day for survival and size, whereas the controls had higher wet weight from day 28 until the day of harvest. Although no Pb-related differences were observed between the intermediate and the final mean values of the Fulton's condition factor calculated for the two treatments (Table 3), a significant negative correlation existed between Pb concentrations (whole body) and this factor ($r = -0.63$, $p < 0.05$).

After 30 d of exposure, we observed abnormal behavior of the exposed organisms, such as surfacing (presumably for air breathing as a response to stress), reduction of motility, and erratic swimming. No behavioral alterations were observed throughout the experiment in the control cultures.

The Pb contents of the different organs and tissues showed a clear trend to increase as a function of time, and the final concentrations in all tissues of the exposed organisms were significantly ($p < 0.05$) higher than the initial values (Table 4). Lead levels in control shrimp also increased, indicating a natural bioaccumulation with regard to age of organism. In addition, the Pb levels in organs and tissues were consistently higher in the Pb-exposed organisms than in controls ($p < 0.05$).

The weight-normalized Pb concentration showed the following order in the final destination of transferred Pb: exoskeleton (28–38% of total Pb) > muscle (23–31%) > remaining tissues of cephalothorax (27–35%) > hepatopancreas (7–11%). Muscle and exoskeleton, which made up approximately 90% of the body mass, contained only between 51 and 69% of the total Pb burden, whereas the remaining organs and tissues contained more than one-third of total Pb, although they made up < 5% of the total body mass.

Haemulon scudderi (level 4)

No significant differences were observed in intermediate and final length, mean wet weight, or Fulton's condition factor throughout the experiment, but final survival was significantly lower in the exposed organisms (Table 5). The behavior of the Pb-exposed and control fish was similar during most of the experiment, but during the last week the exposed fish were more lethargic during feeding, and their food consumption was lower than that of the control fish. In addition, several exposed specimens were observed surfacing, presumably for air breathing.

Table 3. Initial, intermediate, and final mean values of survival, total length (mm), wet weight (g), and Fulton's condition index (K) of *Litopenaeus vannamei* fed the control and the experimental diets (*Artemia franciscana* fed *Tetraselmis suecica* grown in f medium with the background Pb concentration or with 20 μg L^{-1} of Pb)[a]

	Survival (%)		Length (mm)		Wet weight (g)		K	
Day	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
1	100C	100C	6.3 ± 1.6A	6.4 ± 1.1A	1.2 ± 0.3A	1.3 ± 0.2A	0.50 ± 0.1	0.51 ± 0.13
15	94–96C	92–96C	10.8 ± 0.6B	10.5 ± 1.6B	5.1 ± 0.3B	4.8 ± 0.7B	0.48 ± 0.09	0.45 ± 0.17
28	90–95C	88–92BC	13.3 ± 0.6C	12.1 ± 0.6C	7.2 ± 0.4D	6.2 ± 0.3C	0.41 ± 0.13	0.40 ± 0.2
42	84–90B	67–78A	14.8 ± 1.0D	13.1 ± 0.6C	8.5 ± 0.6E	7.1 ± 0.3D	0.38 ± 0.11	0.31 ± 0.14

[a] Results are means ± standard deviation; $n = 12$. Equal letters means no significant differences: A < B < C < D < E.

Table 4. Weighted normalized concentration of Pb (μg g^{-1}) in different organs and tissues of white shrimp *Litopenaneus vannamei* fed with *Artemia franciscana* exposed to Pb via diet[a]

	Exoskeleton		Muscle		Hepatopancreas		Remaining tissues of cephalothorax	
Day	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
0	0.12 ± 0.06A	0.11 ± 0.09A	0.06 ± 0.06A	0.06 ± 0.09A	0.03 ± 0.03A	0.05 ± 0.04A	0.10 ± 0.02A	0.09 ± 0.05A
15	0.46 ± 0.17B	0.67 ± 0.11C	0.21 ± 0.08B	0.33 ± 0.05C	0.14 ± 0.01B	0.19 ± 0.02C	0.12 ± 0.04A	0.48 ± 0.08B
28	0.51 ± 0.09C	0.99 ± 0.14D	0.23 ± 0.11B	0.52 ± 0.12D	0.15 ± 0.09C	0.33 ± 0.05D	0.11 ± 0.04A	0.53 ± 0.11B
42	0.53 ± 0.21C	1.18 ± 0.18E	0.21 ± 0.05B	0.98 ± 0.14E	0.18 ± 0.09C	0.34 ± 0.09D	0.13 ± 0.05A	1.13 ± 0.14C

[a] All results are means ± standard deviation. One-way analysis of variance followed by Tukey's honestly significant difference test was performed for comparing Pb concentrations among groups and treatments. Equal letters means no significant differences: A < B < C < D < E.

Table 5. Initial, intermediate, and final mean values of survival, total length (mm), wet weight (g), and Fulton's condition index (K) of *Haemulon scudderi* fed the control and the experimental diets (*Litopenaeus vannamei* fed *Artemia franciscana* fed *Tetraselmis suecica* grown in f medium with the background Pb concentration or with 20 mg L^{-1} of Pb)[a]

	Survival (%)		Length (mm)		Wet weight (g)		K	
Day	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
1	100D	100D	6.9 ± 1.2A	7.0 ± 0.8A	4.6 ± 1.8A	4.9 ± 1.2A	1.9 ± 0.4	1.9 ± 0.4
15	> 95D	> 90C	7.7 ± 0.2B	7.3 ± 0.2B	7.9 ± 0.2B	6.9 ± 0.8B	1.8 ± 0.3	1.8 ± 0.2
28	88–96C	72–88BC	9.0 ± 1.0C	8.6 ± 0.8C	10.9 ± 2.9C	9.8 ± 1.0C	1.7 ± 0.4	1.7 ± 0.4
42	88–91B	65–75A	10.2 ± 1.0C	9.3 ± 1.0C	15.5 ± 1.0D	14.5 ± 0.8D	1.7 ± 0.4	1.6 ± 0.3

[a] Results are means ± standard deviation; $n = 12$. Equal letters means no significant differences: A < B < C < D < E.

During the experiment, the mean total Pb body burden increased from 0.61 ± 0.08 to 1.24 ± 0.27 μg/g, and from 0.55 ± 0.29 to 3.32 ± 0.41 μg/g in the control and the exposed fish, respectively. The weight-normalized concentration of Pb in different organs and tissues of *H. scudderi* showed a differential accumulation during the exposure period (Table 6). Skeleton, skin, scales, and muscle represented between 82 and 90% of the body mass and accumulated between 70 and 73% of the total metal transferred to this level. Liver and other internal organs and tissues had higher relative contents, indicating a preferential accumulation of Pb in these tissues. The internal organs of exposed fish showed from two to four times more Pb than the controls, with a net contribution close to 30% of total accumulated Pb but with a body dry weight contribution to the total biomass lower than 10%. Small amounts of Pb were accumulated in the gills (~3% of accumulated Pb).

DISCUSSION

Ecotoxicological effects

The effects on the growth rate and cell division observed for *T. suecica* resulting from Pb exposure have been reported previously for different microalgae species, including *Tetraselmis* sp., and for different metals such as Cd [27], Cu [28,29], and Pb [30–33]. The reduced growth rate of *Tetraselmis* and other microalgae exposed to Pb has been explained by these authors as resulting from alterations of metal chelation and phytochelatin production or of enzymatic systems. In particular, Pb in microalgae induces the activity of superoxide dismutase (SOD) isoenzymes, lowers the ratio of reduced to oxidized glutathione (GSH:GSSG ratio), and causes changes in cellular chlorophyll contents generated under stress conditions, which inhibit mitotic spindle formation and consequently inhibit cell division and reduce the growth rate.

In crustaceans, Pb affects hemolymph circulation because of cytological damage such as cytoplasmic vacuolation and reduction of spaces for circulation of the hemolymph [34,35]. This causes breathing difficulties and low internal oxygen concentrations related to impaired hemolymph circulation. In addition, Pb accumulated in organs and tissues may interact with the mitochondrial membranes, decreasing the metabolic rate and consequently O_2 consumption, ammonia excretion, and activity of the enzyme that catalyzes the decomposition of adenosine triphosphate (ATP) [36–38].

We did not observe external morphological anomalies or lesions in the Pb-exposed *H. scudderi*, but some showed difficulty in breathing, lethargy, and reduced consumption of food. The first effect might be due to a Pb-related inhibition of Ca^{2+}, Na^{+}, and Cl^{-} uptake or to cytological damage of the epithelium of the gill tissue [10,11,39], although specific analyses (e.g., number of red blood cell and the activity of δ-aminolevulinic acid dehydratase) and histological evaluations for injuries to this organ and other internal organs, such as liver and kidney, will be required to assess the impact to fish of dietary exposure to Pb.

Comparison with previous studies

As mentioned above, the effects on growth observed for *T. suecica* resulting from Pb exposure have been reported previously for different microalgae species and for different metals [26–29]. However, the grade of growth inhibition varies greatly among different metals, among phytoplankton species, and among experimental conditions (e.g., concentration of metal, initial cell densities, light illumination, temperature,

Table 6. Weight-normalized concentration of Pb ($\mu g\,g^{-1}$) in different organs and tissues of grunt fish *Haemulon scudderi* fed with *Litopenaneus vannamei* exposed to Pb via diet[a]

	Skeleton		Skin/scales		Muscle		Gills		Liver		Viscera (+remain tissues)	
Day	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
0	0.14 ± 0.08A	0.12 ± 0.06A	0.15 ± 0.03A	0.13 ± 0.05A	0.13 ± 0.04A	0.13 ± 0.05A	<LD	<LD	0.11 ± 0.03A	0.10 ± 0.03A	0.07 ± 0.06A	0.06 ± 0.03A
15	0.31 ± 0.09B	0.27 ± 0.05B	0.28 ± 0.08B	0.33 ± 0.1B	0.20 ± 0.02B	0.29 ± 0.10B	0.01 ± 0.02A	0.03 ± 0.02A	0.12 ± 0.05A	0.24 ± 0.02B	0.09 ± 0.05A	0.25 ± 0.08B
28	0.37 ± 0.05B	0.40 ± 0.04B	0.33 ± 0.06B	0.56 ± 0.11C	0.29 ± 0.06B	0.68 ± 0.16C	<LD	0.09 ± 0.01B	0.22 ± 0.04B	0.42 ± 0.06C	0.10 ± 0.05A	0.27 ± 0.03B
42	0.30 ± 0.09B	0.79 ± 0.21C	0.31 ± 0.1B	0.82 ± 0.19C	0.29 ± 0.05B	0.74 ± 0.14C	0.01 ± 0.03A	0.09 ± 0.02B	0.19 ± 0.06B	0.52 ± 0.16C	0.14 ± 0.04A	0.41 ± 0.05C

[a] All results are means ± standard deviation. One-way analysis of variance followed by Tukey's honestly significant difference test was performed for comparing Pb concentrations among groups and treatments. LD = detection limit. Equal letters means no significant differences: A < B < C < D < E.

composition of culture media, and exposure time). Previous experiments with *Tetraselmis* sp. have shown that this genus is relatively more tolerant to metals compared with other microalgae genera. The tolerance of the *Tetraselmis* genus has been related to detoxification mechanisms for sequestering and expelling metal, such as metal chelating complexes, most probably as class III metallothioneins and insoluble salts in the cytoplasm; elevated vacuolization of the cytoplasm; formation of multilayered cell walls; and increase in the excretion of organic matter [27–29,32–34].

Multiple authors have reported negative effects on motility, fecundity and reproduction, and morphological alterations in crustacean species associated with exposure to high levels of Pb (>100 ppb) [8,35–39]. However, previous bioassays with fish exposed to long-term high Pb concentrations (e.g., >100 d to 235 ppb Pb, $\sim$200 d to 120 ppb) have reported a wide range of effects, including neurological degeneration and destruction, lordoscoliosis, paralysis, muscular atrophy, and such morphological alterations as spinal deformities, black tails, and degeneration of the caudal fin [40,41]. Chronic exposure to sublethal Pb levels also causes alteration in physiological functions of fish, such as reduction in food consumption or cessation of feeding, disturbed ionic regulation, reduced swimming speed, growth inhibition, interference with reproduction, and mortality [42,43]. In our short-term exposure with Pb doses lower than those used in most reported studies, none of these effects was verified.

Although the exposed shrimp and fish did not show morphological changes and appeared healthy and active during most of the exposure period, this study concerned only short-term, subtle effects of moderate doses of Pb. Health implications of long-term exposure to moderate doses of Pb are beyond the scope of the present study.

Lead trophic transference

This experiment did not follow the traditional linear responses of aquatic species exposed to unrealistic concentrations of a toxicant for time periods that are short relative to the organism's life span. Rather, our experiment was designed to study the trophic transfer and any biological effects of Pb through a multispecies system covering a four-trophic-level food chain. We exposed the primary producer of our food chain to environmentally realistic concentrations of dissolved Pb; examined its effect on the next trophic levels caused by the consumption of whole, living prey organisms; and quantified the Pb content in different tissues of shrimp and fish.

The trophic transfer of Pb and the biological responses depend on the structure and composition of the aquatic food chain, so the selection of species is a determining factor when studying the effects of a pollutant, because different trophic relations can yield different scenarios for metal transference and variability of responses. This study included some organisms that are common residents of the coastal waters of the southeastern Gulf of California and, for practical reasons, some species that are commercially important. For example, *T. suecica* is widely distributed and is commonly utilized in aquaculture as food for fishes, crustaceans, and mollusks. *Litopenaeus vannamei* is the most widely cultivated shrimp species in the world and grunt fish is a commercially important resource in the region; this issue is of particular relevance for the safety of marine products. *Artemia franciscana* was the only species used that is not a resident in the regional coastal waters, although it is widely used in commercial hatcheries. Among marine invertebrates, *Artemia* is one of the most commonly used species as a test organism to assess toxic effects of metals because of several characteristics that guarantee reliability and feasibility in ecotoxicology studies.

Because *T. suecica* is the basis of our simulated marine food chain, the effects of Pb on the growth and development of phytoplankton are transferred to the zooplankton and subsequently to the upper trophic levels. However, the concentration of Pb did not increase as the metal moved up through the food chain [13]. This suggests mechanisms of self-regulation and elimination of Pb in the species used in our simulated food chain.

Harmful effects in our simulated marine food chain exposed to chronic levels of Pb were not always obvious in the organisms exposed, presumably because they might take more time to appear. However, sustaining a laboratory-based food chain during the entire-life stage is complicated and expensive, which makes entire-life experiments difficult. To the best of our knowledge, no studies following this strategy are available in the literature. In fact, few studies in the literature have related the whole-body Pb concentrations in an organism and concentrations in their tissues and organs to sublethal effects of Pb via diet, which makes it difficult to reach definitive conclusions. Even so, the results revealed that added Pb affected physiological functions and behavior of organisms without killing them.

Ecological implications

A major challenge in this study was to translate the biological response data in terms of ecological significance and possible application under natural circumstances. Our design took into account some of the environmental conditions and interactions in subtropical coastal ecosystems. For this reason, the adverse effects on individual fitness and survival observed in our organisms exposed to Pb via diet probably allow inference of the direct consequences of Pb contamination to the local populations and whole communities.

Eutrophication has been increasing in coastal waters from Gulf of California as a result of inputs of nutrients (C, N, and P) associated with the increase of population living in coastal areas and the rise in demand for foods that require a greater use of fertilizers and animal feed. In addition, the Gulf of California coastal zone is also being influenced by metals inputs from agricultural, mining, industrial, and domestic activities. Although it is known that alteration in the cycling of nutrients in coastal waters provokes perturbations in the biogeochemical cycles of trace metals, knowledge about the influence of nutrient enrichments on the metal uptake in natural ecosystems and, consequently, the toxicity and trophic transfers in aquatic food webs is very limited. For this reason, it is necessary to perform additional studies on the metal transfer processes and on the short- and long-term effects on local species to assess their ecological implications, including their possible consequences for human life.

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