Arch. Environ. Contam. Toxicol. 39, 345–351 (2000)
DOI: 10.1007/s002440010114

ARCHIVES OF
Environmental
Contamination
and Toxicology

Comparative Toxicity of Dissolved Metals to Early Larval Stages of *Palaemon serratus*, *Maja squinado*, and *Homarus gammarus* (Crustacea:Decapoda)

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Received: 24 January 2000/Accepted: 3 May 2000

Abstract. The acute lethal toxicities of mercury, copper, and cadmium to the first larval stage of the prawn (*Palaemon serratus*), spider crab (*Maja squinado*), and lobster (*Homarus gammarus*) were tested. Chromium was also tested with the prawn. Mortality was recorded after 48 h (for lobster) or 72 h (for other species) incubation at 18°C, and the median lethal concentrations (LC_{50} ± 95% confidence intervals) per individual and per mass unit were calculated. The LC_{50} values were, 74 μg Hg/L, 3,304 μg Cu/L, 1,686 μg Cd/L, 12,486 μg Cr/L for prawn; 72 μg Hg/L, 50 μg Cu/L, 158 μg Cd/L for spider crab; and 48 μg Hg/L, 46 μg Cu/L, 34 μg Cd/L for lobster. Therefore, larvae of spider crab and lobster were markedly more sensitive than prawn to heavy metals and thus more suitable to use in seawater quality bioassays. Of these two species, spider crab is recommended due to its abundance, easier maintenance, and higher fecundity. The mercury LC_{50} values for different larval stages of *P. serratus* (zoea I, II, V, and VI) were obtained, and no ontogenetic change in sensitivity to the metal could be detected.

Estuaries are known to accumulate significant amounts of the persistent pollutants transported by continental waters. Metals, in particular mercury (Hg), copper (Cu), cadmium (Cd), and chromium (Cr), are common pollutants in urban and industrial effluents (Young *et al*. 1978; Abel 1989; Kennish 1992). The high toxicity of these metals on early stages of marine invertebrates was previously reported by Okubo and Okubo (1962), Wisely and Blick (1967), Brown and Ahsanullah (1971), Connor (1972), Martin *et al*. (1981), and Beiras and His (1994). Due to their ecological and commercial relevance, crustaceans are frequently used as toxicity test species. Corner and Sparrow (1956) showed that several species of small marine crustaceans were very sensitive to the effect of heavy metals. Other authors have extended these findings to decapod crustaceans: lobsters *Homarus americanus* (McLesse 1974; Johnson and Gentile 1979), prawns *Crangon crangon* (Portmann and Wilson 1971) and *Palaemon serratus* (Wilson and Connor 1971), crabs *Carcinus maenas* (Portmann 1969), and shrimp *Penaeus japonicus* (Bambang *et al*. 1995).

The larval stages of crustaceans are generally more sensitive than adults (*e.g.*, Connor 1972). Ahsanullah and Arnott (1978), studying a species of the genus *Paragrapsus*, claimed that larvae are 9 and 60 times more sensitive than adults to cadmium and zinc, respectively. Rodriguez and Establier (1983) found differences in sensitivity among several larval stages of *Penaeus kerathurus*, with resistance increasing on larval development.

The test species in this study were *P. serratus* (Pennant 1777), *Maja squinado* (Herbst 1778), and *Homarus gammarus* (Linnaeus 1758). These species were chosen using two criteria: abundance and commercial importance. Our aim was to describe the acute toxicity of mercury, copper, and cadmium on the first larval stages by mean of concentration:response curves and calculation of the median lethal concentration (LC_{50}) for each metal. Toxicity data of this kind provide biological criteria to establish seawater quality standards that protect the shellfish resources of the coastal environment. The results of this study should also contribute to the choice of a crustacean species to be included in the bioassays for the assessment of seawater quality.

Materials and Methods

Biological Material

The toxicity tests were performed with the first larval stages; the zoea I of common prawn (*P. serratus*), the zoea I of spider crab (*M. squinado*), and the mysis of European lobster (*H. gammarus*). Mature females were either captured in Ria de Arousa (Galicia, NW Spain) or purchased at the local market and transported to Instituto Galego de Formación en Acuicultura (IGAFA), where the experiments were performed.

The maintenance of the adults in the laboratory and the larval rearing were based on the methodology of Poza (1991). Ovigerous females of *P. serratus* prawn were maintained in open circulating sea water pumped from the sea and filtered through sand and a 0.45-μm filter. The eggs were checked daily to establish the exact day of

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release. Ovigerous individuals were separated into 2-dm^3 individual tanks to obtain individual stocks of larvae hatched from a single female. Larvae were retained on a 300-μm sieve and then transferred into 500-ml glass vessels, vigorously aerated until the beginning of the toxicity test. Ovigerous females of *M. squinado* and *H. gammarus* were collected from the natural environment or purchased on the local market and rapidly transported to the laboratory. The water system was similar to that used for *P. serratus*, but the sea water was filtered by sand only. Females with eggs close to hatching were kept in individual tanks. Therefore, we were able to conduct each test with genetically uniform larvae. Only active swimming larvae were used for testing.

To test older larval stages, newly hatched larvae were fed with nauplii (zoeae I and II) and metanauplii (other stages) of *Artemia salina* (EG-Artemia cysts: Inve Aquaculture). Before feeding larvae, *Artemia* was enriched with a commercial proteinic supplement (Selko), which demands daily water changes. During the weekends for enrichment we used *Isocrysis galvania* and *Tetraselmis suecica* algae rather than Selko because water was not changed daily. The larval culture was held in 2-L plastic cylinders with mesh on the walls to allow water exchange, fitted inside 100-L plastic tanks with flow-through 0.45-μm-filtered sea water. Every 2 days, the dead larvae were removed and food was added at a rate of 50 nauplii or metanauplii per larva. Further details on the methods for maintenance of the adults and larval rearing are described in Mariño-Balsa (1998).

Three samples of 100 larvae (30 larvae for *H. gammarus*) taken from each stock were rinsed with ammonium formate, dried at 70°C in an oven for 24 h, and weighed to 0.0001 g with a Mettler AJ 100 balance to obtain the dry body weight. Samples were then ashed in a Phoenix muffle at 450°C overnight and weighed to obtain the ash-free dry weight, as an estimate of the organic weight (OW).

Incubation Temperature and Density

The optimum temperature of incubation for the larvae was the first parameter studied. Four temperatures were tested: 16°C, 18°C, 20°C, and 22°C, chosen from the high range of environmentally realistic temperatures in the area. All experiments were conducted in four Selecta (model Medilow) incubators with a precision of ±1°C. Newly hatched larvae of *P. serratus* (zoea I stage) were used in this study. Larvae were usually released overnight and temperature tolerance tests began in the morning. Twenty-five-milliliters polypropylene vials filled with 20 ml of 0.45-μm-filtered sea water were used for incubation. Ten larvae were selected with a plastic pipette and delivered into each vial. Five vials (replicates) per temperature were set up, therefore 200 larvae were necessary. Larvae were observed *in vivo* by using a binocular microscope Olimpus SZ 40 at 24 h, 48 h, 72 h, and 96 h after the beginning of the test, and the number of the dead larvae per replicate was recorded.

The effect of temperature on larval survival was tested with a general linear model for repeated measures by using the Pillai-Barlett statistic, one of the most robust and powerful commonly utilized for MANOVA (Johnson and Field 1993). Temperatures that caused significant differences in mortality were identified by a *post hoc* Tukey test (Sokal and Rohlf 1981).

The effect of incubation density on larval viability was then assessed using three different densities—10, 15, and 20 larvae per vial—and 18°C as incubation temperature. The methods employed were the same as those described for the temperature trial described above. The results were analyzed by one-way ANOVA.

Chemical Analyses

Metal concentrations in the water were checked for each metal in samples taken at the beginning of the test. The electrochemical method of analyses used for Cu and Cd were the anodic stripping voltammetry (ASV) as described by Fernández *et al*. (1992). A hanging-drop mercury electrode (HMDE), a reference electrode of Ag/AgCl (KCl sat.), and an auxiliary electrode of Pt were employed. The potentiostat was an Eco-Chemie PGSTAT10 fited on a Metrohm VA Stand 663. The determination of metals was made with a dilution 1:1,000 of the original samples. The coefficient of variation of determination was 5% for Cd and 2% for Cu. For Hg, measurements were carried out in 250-ml samples following the technique of cold vapor atomic absorption after gold amalgamation step automated by means of a Flow Injection System (Perkin-Elmer FIAS 200), according to Weltz and Schubert-Jacobs (1991).

Toxicity Tests

Comparative toxicity was studied for Hg, Cu, and Cd using zoea I larvae of *P. serratus* and *M. squinado* and mysis I larvae of *H. gammarus*. Chloride salts of Hg, Cu (Merck, pro-analysis), and Cd (Merck, extra-pure) were employed, but concentrations were always referred to as metallic ion. A Mettler AJ 100 balance with a precision of 0.0001 g was utilized for preparing the stock solutions in volumetric flasks filled with distilled water. Selected experimental concentrations were made up by addition of adequate volumes of the stock solution to 0.45-μm-filtered sea water. The concentrations of the stocks were calculated to avoid diluting the salinity in the vials more than 10%, which was innocuous to these animals (Charmantier *et al*. 1988). Ten active larvae were delivered by pipette into each polypropilene vial of 20 ml (except for larger lobster larvae, where 100-ml polystyrene vessels were used). The experiments were conducted at 18°C and a daily cycle of 14 h light:10 h dark. Dead larvae were counted at 24 h, 48 h, 72 h, and 96 h. After studying the pattern of mortality increase with time in each species, we calculated the LC_{50} after 72 h incubation for the prawn and spider crab larvae and after 48 h incubation for the lobster larvae. In the case of the prawn, several (three to five) independent tests with larvae from different females were conducted with each metal, and the geometric mean of the LC_{50} values (± SD) was also calculated. Larval availability limited the spider-crab and lobster studies to a single test per metal.

In the first test for each metal samples of 150 ml per concentration were taken for chemical analysis. These samples were fixed with hydrochloric acid (Merck suprapur) at 1–2 pH. Samples of Cu and Cd solution were stored in polypropylene flasks; for mercury sampling, teflon flasks were employed.

For mercury, the experimental concentrations initially tested were 16, 64, 256, and 1,024 μg/L plus a control, and in the following tests: 16, 32, 64, 128, and 256 μg/L plus a control. For copper, the first test with prawn consisted of concentrations in a geometric progression 10× (0 [control], 10, 100, 1000, 10,000 μg/L), the next one in progression 4× (0 [control], 100, 400, 1,600, 6,400 μg/L), and finally in progression 2× (0 [control], 400, 800, 1,600, 3,200, 6,400 μg/L). In *M. squinado* and *H. gammarus* the concentrations employed were in both cases: 0 (control), 5, 25, 100, 400, and 1,600 μg/L. For cadmium, in the prawn tests the concentrations were 0 (control), 800, 1,600, 3,200, 6,400, and 12,800 μg/L. In the tests with the other more sensitive species, the concentrations were 0 (control), 5, 25, 100, 400, and 1,600 μg/L. In the case of prawn, the toxicity of chromium was also investigated by testing the concentrations 0 (control), 4,000, 8,000, 16,000, 32,000, and 64,000 μg/L.

To calculate the LC_{50} values, the experimental data were previously corrected taking into account the mortality in the controls, following the expression (Emmens 1948):

$$p = (p_O - p_C)/1 - p_C$$

Where p_O and p_C are, respectively, the proportion of mortality in the treatment and in the control; p is the corrected value.

The LC_{50} and its 95% confidence intervals were calculated by the Litchfield-Wilcoxon method (Newman 1995), following the expressions:

$$\text{Upper 95\% confidence interval} = LC_{50} * fCL_{50}$$

$$\text{Lower 95\% confidence interval} = LC_{50}/fCL_{50}$$

$$f_{LC50} = S^{2.77/(N')^{1/2}}$$

$$S = ((LC_{84}/LC_{50}) + (LC_{50}/LC_{16}))/2$$

N' = Number of total individuals between 16% and 84% of the answer.

LC_{84} and LC_{16} are the concentrations causing 84% and 16% mortality.

Ontogenetic Variation of Sensitivity of P. serratus *to Hg*

The potential ontogenetic change in sensitivity to mercury in *P. serratus* prawn larvae was investigated. The effect of this metal was tested on four larval stages—zoea I, zoea II, zoea V, and zoea VII—at 16, 32, 64, 128, and 256 μg/L plus a control. To establish what extent size increase explains the ontogenetic change in sensitivity, the LC_{50} is considered as a power function of OW^b, according to the allometric equation $LC_{50} = a\ OW^b$, or its linear equivalent, $\log LC_{50} = a' + b \log W$ (Beiras and His 1994). Slopes > 1 indicate a relative increase in resistance (per unit weight), slopes between 1 and 0 indicate a relative decrease in resistance, slopes < 0 indicate an absolute decrease in resistance (per individual) with development.

Results and Discussion

Larval Weight

Prawn larvae grow from approximately 65 μg at release up to approximately 275 μg at zoea VI stage, but the organic content remain constant near 85%. Spider crab larvae are two times heavier at release (125 μg), while lobster larvae are much larger and weigh approximately 30 times more (2,175 μg), with organic contents of 66% and 74%, respectively.

Incubation Temperature and Density

The MANOVA applied to the data for survival of *P. serratus* larvae at different temperatures showed a highly significant effect ($p < 0.001$) of this variable. The results of the *post hoc* Tukey's test are shown in Table 1. After 72 h, larvae held at 22°C showed significantly ($p < 0.001$) lower survival that those at 16°C, 18°C, and 20°C. After 96 h the two highest temperatures showed significantly ($p < 0.001$) increased mortality compared to 16°C and 18°C. Therefore, we chose 18°C as the temperature of incubation for the subsequent experiments. This temperature was also adequate to obtain high and reliable rates of survival for the other two species in the controls.

Table 1. Results of the Tukey's test of multiple comparisons among the survival data for *P. serratus* at 16°C, 18°C, 20°C, and 22°C after 24 h, 48 h, 72 h, and 96 h incubation

	24 h				48 h				72 h				96 h			
	16°C	18°C	20°C	22°C	16°C	18°C	20°C	22°C	16°C	18°C	20°C	22°C	16°C	18°C	20°C	22°C
16°C	—	0.509 NS	1.000 NS	1.000 NS	—	0.808 NS	1.000 NS	0.314 NS	—	0.959 NS	0.213 NS	0.000***	—	0.907 NS	0.000***	0.000***
18°C		—	0.509 NS	0.509 NS		—	0.808 NS	0.808 NS		—	0.433 NS	0.000***		—	0.000***	0.000***
20°C			—	1.000 NS			—	0.314 NS			—	0.000***			—	0.895 NS
22°C				—				—				—				—

NS, not significant at $\alpha = 0.05$.

Table 2. Nominal and measured concentrations (μg/L) of metals in the experimental vessels

Mercury			Copper			Cadmium		
Nominal	Measured	%	Nominal	Measured	%	Nominal	Measured	%
16	16	100	400	360	90	400	463	116
32	33	103	800	680	85	800	804	101
64	64	100	1,600	1,585	99	1,600	1,618	101
128	120	94	3,200	3,210	100	3,200	3,244	101
256	260	102	6,400	6,150	96	6,400	6,455	101

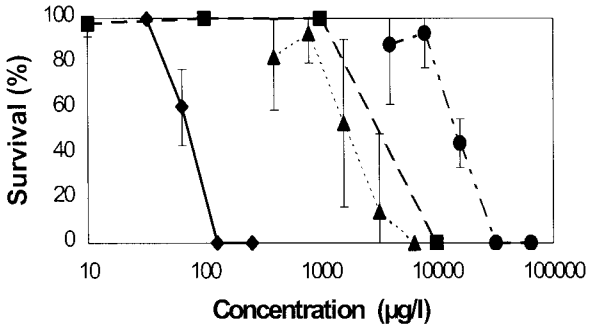

Fig. 1. Concentration:response curves showing the survival rate (%) of *Palaemon serratus* newly released larvae after 72 h exposure to different concentrations (μg/L) of dissolved mercury (diamonds), copper (squares), cadmium (triangles), and chromium (circles)

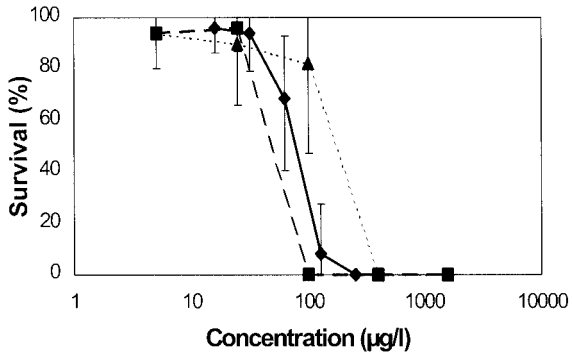

Fig. 2. Concentration:response curves showing the survival rate (%) of *Maja squinado* newly released larvae after 72 h exposure to different concentrations (μg/L) of dissolved mercury (diamonds), copper (squares), and cadmium (triangles)

A one-way ANOVA showed no significant ($p = 0.091$) differences in larval mortality among the densities studied—10, 15, and 20 larvae per 20 ml. Mortality was in all cases lower than 3% until 72 h, and lower than 30% at the end of the experiment (96 h). To maximize statistical power, 20 larvae per vessel could be used, since no increase in mortality was detected. However, the experimental design is still limited by the number of larvae released per female and by the time necessary to pipette and observe each individual, which is necessary to check larval activity before and after incubation. These limitations prevented use of densities higher than 10 per vessel. Given the experimental design used, *i.e.*, five replicates per treatment and 10 larvae per replicate, and a level of significance ($1 - \alpha$) of 95%, we can calculate the minimum detectable difference (δ) and the power of the test ($P = 1 - \beta$) following the equation by Zar (1984):

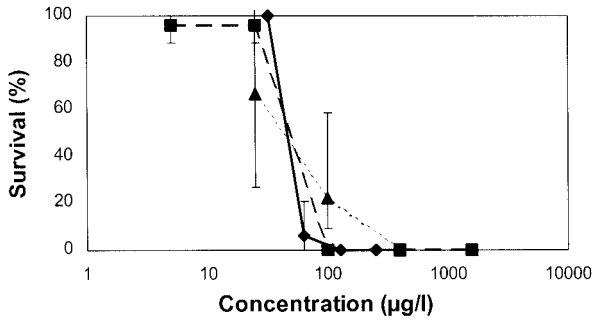

Fig. 3. Concentration:response curves showing the survival rate (%) of *Homarus gammarus* newly released larvae after 48 h exposure to different concentrations (μg/L) of dissolved mercury (diamonds), copper (squares), and cadmium (triangles)

$$n \geq 2S_p^2/\delta^2(t_{\alpha,\upsilon} + t_{\beta(1),\upsilon})^2$$

where n is the minimum sample size, S_p^2 is the variance, and υ is the degrees of freedom. The results were: $P = 0.8$ and $\delta = 15\%$ mortality. Therefore, our experimental design is able to detect true differences in mortality as low as 15% in 80% of the cases.

Exposure Time

The effect of exposure time on toxicity depended on the test species. In *P. serratus* and *M. squinado*, mortality was negligible at 24 h, whereas in *H. gammarus* there was already some mortality at the highest concentration after 24 h. However, toxic effects were not significant until 48 h. Prolonging exposure for 1 more day did not increase the mortality further (data not shown). Therefore, LC_{50} for *H. gammarus* was calculated at 48 h. In contrast, for *P. serratus* and *M. squinado*, mortality did increase after 72 h exposure, and this period was chosen for LC_{50} calculation.

Chemical Analyses

Table 2 shows the results of the analytical chemistry applied for checking the nominal concentrations. Actual concentrations obtained in the experimental vessels ranged from 85 to 116%

Table 3. Median lethal concentration (LC_{50}; μg/L and μg/L per μg of organic weight [OW]) in 1-day-old larvae of *P. serratus*, *M. squinado*, and *H. gammarus*. Exposure time was 48 h for *H. gammarus* and 72 h for *P. serratus* and *M. squinado*

	Hg			Cu			Cd		
Species	LC_{50} (μg/L)	95% CI	LC_{50} (μg/L μgOW)	LC_{50} (μg/L)	95% CI	LC_{50} (μg/L μgOW)	LC_{50} (μg/L)	95% CI	LC_{50} (μg/l μgOW)
P. serratus	43	(32; 59)		3.160	NC		1,739	(1,528; 1,980)	
	105	(89; 123)		5.751	(4,009; 8,250)		1,864	(1,600; 2,173)	
	56	(48; 65)		1.985	(1,703; 2,315)		1,479	(1,272; 1,719)	
	138	(111; 173)							
	66	(58; 75)							
	74 ± 39		1.28	3,304 ± 1,927		57.27	1,686 ± 196		29.23
M. squinado	72	(60; 87)	0.88	50	(46; 55)	0.61	158	(133; 188)	1.94
H. gammarus	48	NC	0.03	46	NC	0.03	34	(25; 97)	0.02

NC, not calculable by the Litchfield-Wilcoxon method.

of the desired nominal concentrations, thus discounting any important loss of metal by adsorption or evaporation.

Toxicity Tests

Figures 1 to 3 show the concentration:response curves with the survival rates obtained for the different metals with newly released larvae of *P. serratus* prawn (Figure 1), *M. squinado* spider crab (Figure 2), and *H. gammarus* lobster (Figure 3). Table 3 shows the LC_{50} of mercury, copper, and cadmium on these larvae. For chromium, the LC_{50} value obtained with prawn larvae was very high (12,486 ± 1,683 μg/L, mean of three tests and SD), and we did not use this element in further trials.

The metals studied can be ordered according to their toxicity to prawn larvae as: Hg ≫ Cd > Cu > Cr. In *P. serratus*, mercury was more than one order of magnitude more toxic than the other two metals. Considering the inherent biological variability of the larval stocks used for the different tests, the results for each metal can be regarded as consistent. However, the coefficients of variation for the mean LC_{50} of all tests with prawn (52%, 63%, 12%, for Hg, Cu, and Cd respectively) show moderately high values for the two first metals and lower interstock variability for cadmium toxicity.

In *M. squinado* and *H. gammarus* only one test was conducted due to limited larval availability for these species. Mercury again exhibited very high toxicity, with values of LC_{50} below 100 μg/L, but in contrast to *P. serratus*, spider crab and lobster larvae were markedly more sensitive to copper and cadmium. For the spider crab, the relative toxicities were Cu > Hg > Cd, and for the lobster, Cd > Hg ≈ Cu. It is important to emphasize the low LC_{50} obtained for cadmium in lobster larvae, 34 μg/L.

Mercury was the only metal that showed toxicity under the 100 μg/L level in the three species. This finding agrees with the literature of metal toxicity to decapod larvae (Connor 1972; Johnson and Gentile 1979; Martin *et al*. 1981; Rodriguez and Establier 1983; Mortimer and Miller 1994; Bambang *et al*. 1994).

The toxicity of metals on *P. serratus* larvae was previously studied by Le Dean and Devineau (1987), who found a lower LC_{50} for cadmium (220–450 μg/L), probably due to the longer exposure period (10 days). Time of exposure is a crucial parameter when obtaining standard measures of LC_{50}. Many studies use 48 h as standard exposure time (Connor 1972; Shealey and Sandifer 1975; Wong *et al*. 1993, 1995; Mortimer and Miller 1994; Ramachandran 1997; present study with lobster) However, Glickstein (1978) showed that Hg LC_{50} decreased from 21 μg/L to 7 μg/L as exposure time increased from 48 h to 96 h, and Bambang *et al*. (1994) found a decrease in Cd LC_{50} from 240 μg/L at 48 h down to 10–30 μg/L at 96 h. We have also found a marked decrease in LC_{50} from 48 h to 72 h in *P. serratus* and *M. squinado*. The present experimental design, with no food added, showed significant mortality in the controls at longer incubation times. Other authors, though, chose 96 h as standard exposure time with (Johnson and Gentile 1979; Martin *et al*. 1981) or without feeding the larvae (Ahsanullah and Arnott 1978).

To the best of our knowledge, this study presents the first account on metal toxicity to larvae of a member of the family Majidae. With respect to the lobster, our results agree with those from Connor (1972), who reported LC_{50}s of 33–100 μg Hg/L and 100–330 μg Cu/L, and Johnson and Gentile (1979), the latter with the American lobster *H. americanus*, with LC_{50}s of 20 μg Hg/L, 48 μg Cu/L, and 78 μg Cd/L.

Environmental risk assessment often requires prediction of potential toxic effects in the long term from acute toxicity data to implement maximum admissible concentrations of pollutants in the water. According to the review from Länge *et al*. (1998), the 90th percentile of the distribution of the ratios between the levels of pollutants causing acute and chronic toxicity in a broad range of aquatic organisms was 73. These data support the use of a protection factor of 100 to obtain maximum permissible concentrations (MPCs) from acute LC_{50} values as MPC = LC_{50}/100.

Ontogenetic Variation of Sensitivity of P. serratus *to Hg*

For zoea stages II, V, and VI, the LC_{50} values (μg/L) were 55 (48, 64), 36 (31, 42), and 56 (47, 68), respectively (95% confidence intervals in parentheses). Based on the average organic weight per larva at each developmental stage, the

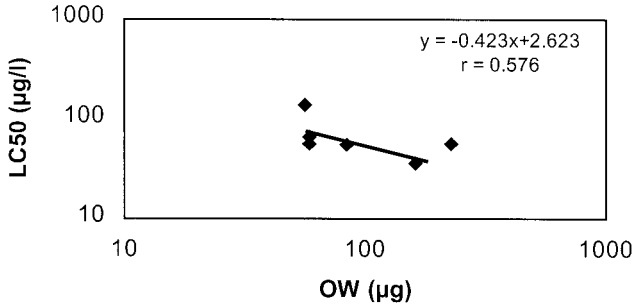

Fig. 4. Variation of the median lethal concentration (LC_{50}, µg/L) of dissolved mercury as a function of the organic weight (OW, µg) in different larval stages of *Palaemon serratus* prawn larvae

organic weight–specific LC_{50} values for zoea I, II, V, and VI were 1.28, 0.66, 0.22, and 0.24 µg Hg/µg OW, respectively.

The general pattern observed in marine invertebrate larvae consists of a decrease in larval sensitivity to toxicants with age (*e.g.*, Rodriguez and Establier 1983; Wong *et al*. 1993; Bambang *et al*. 1994; Beiras and His 1994, 1995). Rodriguez and Establier (1983) found an increase in resistance along larval development of *Penaeus kerathurus*. In contrast, DeCoursey and Vernberg (1972) claimed that the toxicity of mercury increases as larval size increases in *Uca pugilator*.

The present results show an increase of larval sensitivity to mercury as larval development progresses. When the LC_{50} was modeled as a power function of OW (Figure 4), the resultant equation was $\log LC_{50} = 2.623 - 0.423 \log OW$ ($r = -0.576$, $n = 6$). As the low correlation coefficient indicates, very little variability is explained by the model. Moreover, the 95% confidence intervals of the slope (−0.845, 0.392) included 0, indicating that the negative slope is not significant. Therefore, in the present study, there is no evidence of a change in sensitivity with larval development.

Conclusions

Of the four metals studied, mercury was the most toxic, with an average LC_{50} of 65 µg/L for the three species studied. Prawn (*P. serratus*) larvae were markedly more resistant to copper and cadmium than spider crab (*M. squinado*) and lobster (*H. gammarus*) larvae. The lobster was the most sensitive species to heavy metals, with a Cd LC_{50} of 34 µg/L, the lowest for all species–metal combinations studied. Despite slightly higher sensitivity of lobster, the spider crab is considered the preferable choice for seawater quality bioassays with crustacean larvae due to greater abundance of adults, easier maintenance in captivity, higher number of larvae per female, and other characteristics of its reproduction.

Based on the limited biological criteria obtained in the present study with decapod species and using a protection factor of 100 as previously discussed, maximum permissible concentrations would be of the following order: mercury, 0.5 µg/L; copper, 0.5 µg/L; cadmium, 0.3 µg/L; and chromium (based on data for prawn larvae only), 100 µg/L. According to the literature, metal concentrations in coastal areas may occasionally exceed these levels. This stresses the need for the inclusion of crustacean species in the biological assessment of the marine environment.

Acknowledgments. We would like to thank all the staff at IGAFA (Xunta de Galicia) for enabling and collaborating in the experimental work of this study. Chemical analyses were performed by A. Cobelo from the Analytical Chemistry Department (University of Vigo), and J. J. González and J. Fumega from the IEO-Vigo. This work was funded by Spanish CITMAR (Project MAR96-1824).

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