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Effects of dissolved mercury on embryogenesis, survival and growth of *Mytilus galloprovincialis* mussel larvae

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ABSTRACT: The effects of Hg concentrations ranging from 0 (control) to 1024 µg l^{-1} upon embryogenesis, survival and growth of *Mytilus galloprovincialis* mussel larvae were investigated. Embryogenesis was abnormal in 50% of the individuals at 10 µg l^{-1}. The 48 h LC_{50} for D-shaped, early umbonate, late umbonate and eyed larvae were 51, 164, 322 and 383 µg l^{-1} respectively. The LC_{50} was an allometric function of ash-free dry weight with exponent b = 0.60. Larval growth was significantly reduced after 6 d exposure to 4 µg Hg l^{-1} and after 10 d exposure to 2 µg l^{-1}. No significant differences in lethal or sublethal sensitivity to Hg were found between *M. galloprovincialis* and previously studied *Crassostrea gigas* embryos and larvae. The implications of these findings for monitoring pollution by utilising different bivalve species are discussed.

KEY WORDS: Mercury · *Mytilus* · Mussel · Larva · Growth · Embryo · Bioassay · Ecotoxicology

INTRODUCTION

The first developmental stages of bivalves have been shown to be highly sensitive to micropollutants, particularly to heavy metals (Brereton et al. 1973, Calabrese et al. 1973, 1977, Calabrese & Nelson 1974, His & Robert 1981, 1982, Martin et al. 1981, Thain 1984). Therefore, oyster embryos and larvae have been increasingly used in bioassays to provide biological criteria for marine sediment and water quality assessment (e.g. Chapman & Morgan 1983, Butler et al. 1992, Beiras & His 1995). High toxicity and bioaccumulation give Hg a well-known ecotoxicological relevance among the heavy metals (reviewed by Cossa et al. 1990). *Crassostrea gigas* (Thunberg) embryogenesis was found to be abnormal in 50% of the individuals at 11 µg Hg l^{-1}, and larval growth significantly retarded at 4 µg l^{-1} (Beiras & His 1994).

The mussel *Mytilus galloprovincialis* Lamarck is a major component of the littoral fauna in the Mediterranean Sea and along the Atlantic coasts of Spain, France and Great Britain. It shows a long natural spawning season from late winter to late summer, and is easily available due to its commercial importance. The aim of this investigation was to assess both the lethal and sublethal (growth) effects of Hg on *M. galloprovincialis* embryos and larvae, and to compare the sensitivity of mussel larvae to oysters, in order to evaluate the potential use of *M. galloprovincialis* as an alternative or complementary species in ecotoxicological studies.

MATERIAL AND METHODS

General methods concerning seawater treatment, fertilization, embryogenesis and larval rearing were described elsewhere (Beiras & His 1994).

Fresh stock solutions of 10 mg ionic Hg^{2+} l^{-1} were prepared from $HgCl_2$ (analytical grade) and distilled water within 24 h of the start of the experiments. Filtered (0.2 µm) natural seawater of oceanic characteristics (35 ppt salinity) from the Bassin d'Arcachon, France, was used for the experiments. Maximum reduction in salinity due to Hg stock solution addition was 10.2% (i.e. 31 ppt), within the range of optimal

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salinities for the early development of *Mytilus galloprovincialis* (His et al. 1989). All glassware was acid-washed (HNO_3 10% vol.), rinsed with distilled water and sterilized at 170°C for 2 h before the experiments.

For embryogenesis experiments, fertilized eggs were transferred to 25 ml polyethylene accuvettes (4 or 5 replicates per treatment) filled with the following nominal Hg concentrations: 0 (controls), 2, 4, 8, 16, 32 and 64 µg l^{-1}. The accuvettes were then capped and incubated at 20 ± 1°C for 48 h. After incubation, buffered formalin was added to each accuvette and a minimum of 100 individuals per accuvette were observed under an inverted microscope in order to record the number of abnormal larvae.

For larval survival experiments, the Hg concentrations tested ranged from 2 to 1024 µg l^{-1}, increasing in a $\log_2$ scale, plus a control. The accuvettes were microscopically observed after 24 and 48 h prior to formalin addition in order to record *in vivo* the larval appearance and swimming behaviour. For each developmental stage, larval height and length were measured using a graduated graticule on a sample n = 50. Samples of a known number of larvae from nonexposed stocks were taken to obtain individual dry weight and ash-free dry weight (AFDW) according to the procedures described by Beiras & Pérez Camacho (1994).

The Hg concentrations that caused 50% abnormalities in embryogenesis, AC_{50}, and 50% mortality, LC_{50}, were calculated as previously described (Beiras & His 1994).

For larval growth experiments the Hg concentrations tested were 0 (controls), 1, 2, 4, 8 and 16 µg l^{-1}, and the methods described by Beiras & His (1994) for oyster larvae were also followed, except that mussel larvae were reared at 20 ± 1°C, the optimal temperature for this species (His et al. 1989). The average growth rates (GR, µm d^{-1}) were calculated as $GR = (H_t - H_0)/t$, where H_0 = initial height, H_t = final height and t = time.

Regression analyses were performed by using Statgraphics computer software (Manugistics 1993). Comparison of slopes of linear regression equations was performed according to Zar (1974).

RESULTS

Embryogenesis and larval survival

A consistent increase in tolerance to Hg with age was noted (Table 1). The AC_{50} for embryos was 10 µg l^{-1} and the LC_{50} were 51, 164, 322 and 383 µg l^{-1} for D-shaped, early umbonate, late umbonate and eyed larvae respectively. The ln LC_{50} was found to be a linear function of ln ash-free dry weight (AFDW, µg) (Fig. 1), i.e. increasing larval tolerance to Hg could be explained by its organic weight increase following the equation (± SE):

$$\ln LC_{50} = 5.81\ (\pm 0.081) + 0.60\ (\pm 0.049)\ \ln AFDW,$$
$$r = 0.994,\ p = 0.006.$$

Table 1. *Mytilus galloprovincialis*. Mean (n = 4 to 5) percentage abnormalities for embryos and mean percentage mortality for D-shaped, umbonate and eyed larvae exposed to different Hg concentrations for 48 h. The 95% confidence intervals are given in parentheses. Calculations made using angular-transformed percentages but values were back-transformed for presentation. Height (mean SD; n = 50) of larvae is also shown. Larval swimming activity was classified arbitrarily as normal, reduced or null. nm: not measured

	Hg concentration (µg l^{-1})										
	0	2	4	8	16	32	64	128	256	512	1024
Embryos	6.2 (4.28,8.18)	6.1 (3.06,9.63)	5.9 (1.85,10.80)	10.8 (7.45,14.39)	99.2 (97.86,99.99)	100	100	nm	nm	nm	nm
D-shaped (76 ± 3.2 µm) swimming	1.2 (0.03,3.32) normal	1.2 (0.33,3.41) reduced	0.4 (0.08,1.17) reduced	1.2 (0.06,2.83) reduced	3.1 (0.06,7.57) reduced	1.4 (0.54,2.42) null	85.0 (79.11,90.38) null	97.6 (93.45,99.95) null	nm	nm	nm
Umbonate I (141 ± 17.0 µm) swimming	3.1 (0.14,7.42) normal	nm	0.4 (0.08,1.17) normal	1.2 (0.06,3.38) normal	2.0 (0.55,3.79) normal	3.8 (2.21,5.57) normal	4.5 (0.59,9.64) normal	33.6 (11.67,52.13) reduced	74.0 (34.80,99.89) null	nm	nm
Umbonate II (225 ± 19.7 µm) swimming	4.2 (0.61,8.91) normal	nm	nm	6.8 (0.47,15.38) normal	3.0 (0.08,7.29) normal	5.4 (1.71,9.84) normal	7.0 (4.32,9.94) reduced	5.4 (2.47,8.78) null	28.0 (12.19,45.74) null	86.2 (72.81,97.21) null	98.4 (95.52,99.93) null
Eyed (258 ± 18.7 µm) swimming	10.7 (7.80,13.81) normal	nm	nm	nm	11.1 (8.15,14.40) normal	17.1 (9.94,25.30) normal	18.2 (7.22,31.47) reduced	17.8 (1.91,41.13) reduced	19.9 (8.43,33.68) null	71.1 (66.61,71.55) null	99.0 (96.03,99.58) null

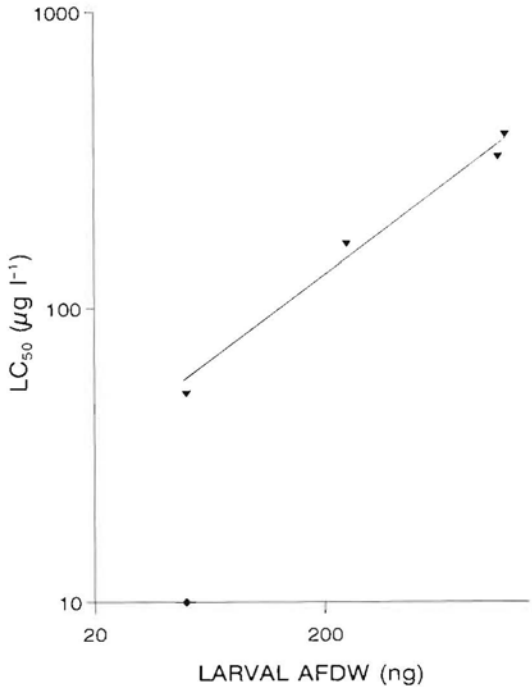

Fig. 1. *Mytilus galloprovincialis*. Double logarithmic plot relating Hg concentration causing 50% larval mortality after 48 h (LC_{50}) to larval ash-free dry weight (AFDW). (♦) Hg concentration causing 50% abnormalities in embryogenesis (AC_{50}) assuming no AFDW change during the endotrophic development

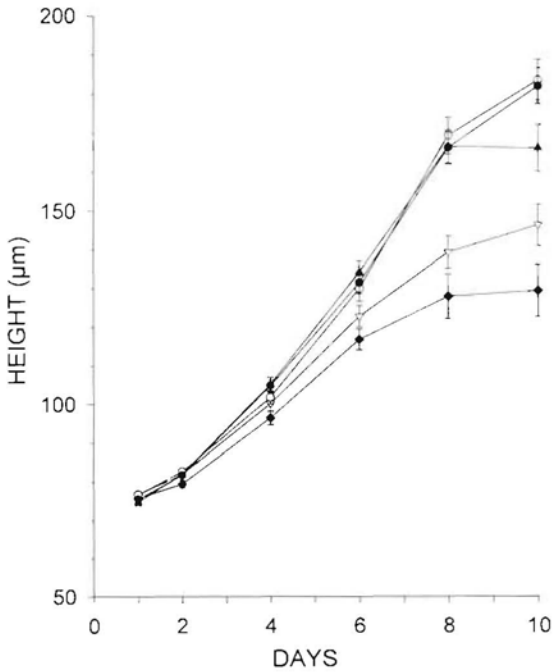

Fig. 2. *Mytilus galloprovincialis*. Height increase in larvae reared from D-shaped stage at 0 (●), 1 (○), 2 (△), 4 (▲) and 8 (♦) µg Hg l^{-1} over 10 d. Error bars: ±SE

Growth

The allometric growth of *Mytilus galloprovincialis* larvae is described by the following equation:

$$H = 0.244\ (\pm 1.0338)\ L^{1.227\ (\pm 0.0062)},$$
$$r = 0.997,\ n = 200$$

where H = height (µm) and L = length (µm).

Shell height increase is presented in Fig. 2 for the different Hg concentrations. Size was significantly ($p < 0.05$) reduced at 4 and 8 µg l^{-1} from Day 6, and at Hg concentrations >1 µg l^{-1} at Day 10. While size increase in nontoxic treatments fitted well to a straight line, deviations from linear growth were found at the effective Hg concentrations, as expected, considering that toxicity is directly related to exposure time (Hayes 1991). A plateau seemed to have been reached the last 2 d of experiments by the larvae exposed, to 2, 4 and 8 µg Hg l^{-1}. This prevented us from fitting straight lines. Rather, growth retardation was further investigated relating average growth rate (GR, µm d^{-1}) to Hg concentration (C, µg l^{-1}). The following highly significant regression model was obtained (20°C, 35 ppt):

$$GR = 11.9\ (\pm 0.50) - 0.80\ (\pm 0.121)\,C,$$
$$r = -0.967,\ p = 0.007.$$

DISCUSSION

No major interspecific differences in embryonic sensitivity to Hg have been found among several bivalve species, notwithstanding variability due to different studies, especially considering that calculations were made using nominal concentrations. Reported AC_{50} values (µg Hg l^{-1}) were 10 in *Mytilus galloprovincialis*, 6 to 32 in *Mytilus edulis*, 7 to 32 in *Crassostrea gigas* and 4 to 7 in *Crassostrea virginica* (Okubo & Okubo 1962, Calabrese et al. 1973, Martin et al. 1981, Beiras & His 1994, present study). When only data from the same laboratories are considered to ensure comparable methodology, an even greater similarity in sensitivity to Hg can be found. The AC_{50} was 6.7 for *C. gigas* and 5.8 for *M. edulis* in the study by Martin et al. (1981), and 13 for *C. gigas* and 10 for *M. galloprovincialis* in our studies (Beiras & His 1994, present study). Similarly, Calabrese and coworkers in independent experiments reported AC_{50} values of 5.6 and 4.8 µg Hg l^{-1} for *C. virginica* and *Mercenaria mercenaria* embryos respectively (Calabrese et al. 1973, Calabrese & Nelson 1974). Watling (1982) also found almost equal sensitivity to copper among embryos and larvae of 3 *Crassostrea* spp. Roberts (1987) stated that there clearly was no significant difference in LC_{50} between clam (*M. mercenaria*) and

oyster (*C. virginica*) embryos or clam and oyster larvae exposed to tributyltin.

Comparisons among different studies on larvae are limited by different exposure times, larval rearing conditions and particularly by different larval sizes (frequently not shown), since larger larvae are expected to be more resistant to pollutants. Nevertheless, we have previously found (Beiras & His 1994) that there is an increase during the ontogenic development of *Crassostrea gigas* in the weight-specific sensitivity to Hg, characterized by an exponent $b < 1$ in the allometric relationship LC_{50} vs AFDW. New results are presented here extending this finding to *Mytilus galloprovincialis*, where $b = 0.60 \pm 0.049$ (± SE), significantly lower than 1, i.e. the LC_{50} per unit of weight is lower in larger larvae. Differences in sensitivity between both species were further investigated taking into account the effect of larval size by comparing the fitting parameters of the LC_{50} vs AFDW regressions. The slope was higher for *M. galloprovincialis* than for *C. gigas* (b = 0.46; Beiras & His 1994) suggesting that larger mussel larvae might become slightly more resistant to Hg than oyster larvae of equal size; however, these differences were not statistically significant ($F = 6.99$; $F_{1,3,0.05} = 10.13$).

While genetic homogeneity of the test organisms is a theoretical requirement for comparing toxicity data, experimental evidence within bivalves demonstrates a limited interspecific variability in embryo-larval sensitivity to heavy metals (Martin et al. 1981, Watling 1982, Roberts 1987, Beiras & His 1994, present study). Moreover, a sublethal response to Hg exposure, i.e. the decrease in larval growth rate (*GR*), was also not significantly different between *Mytilus galloprovincialis* (slope of the *GR*:Hg concentration regression, $b = -0.80 \pm 0.121$) and *Crassostrea gigas* ($b = -0.92 \pm 0.071$; Beiras & His 1994). These results encourage the complementary use of *C. gigas*, *M. galloprovincialis* and other bivalve species for routine embryo-larval toxicity bioassays, thus enabling toxicity testing over a wider geographic and seasonal range. In addition, similar sensitivity to chemical stress is not unexpected considering the common ecological role played by the veliger larvae of most bivalves as members of the zooplankton.

Inhibition of swimming was observed here (Table 1) and in our previous study (Beiras & His 1994) at very low sublethal Hg levels. The potential use of this sensitive response applied to the bivalve larval bioassay is the subject of current investigation in our laboratory. The study of increasingly sensitive sublethal responses is intended to close the gap between the effective micropollutant concentrations found in laboratory experiments and the ecologically relevant concentrations available in the marine environment.

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