

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

R. Beiras*, E. His

Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Station d'Arcachon, Quai Silhouette, F-33120 Arcachon, France

ABSTRACT: The effects of Hg concentrations ranging from 0 (control) to 1024 $\mu\text{g l}^{-1}$ upon embryogenesis, survival and growth of *Mytilus galloprovincialis* mussel larvae were investigated. Embryogenesis was abnormal in 50% of the individuals at 10 $\mu\text{g l}^{-1}$. The 48 h LC_{50} for D-shaped, early umbonate, late umbonate and eyed larvae were 51, 164, 322 and 383 $\mu\text{g l}^{-1}$ 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 $\mu\text{g Hg l}^{-1}$ and after 10 d exposure to 2 $\mu\text{g l}^{-1}$. 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 $\mu\text{g Hg l}^{-1}$, and larval growth significantly retarded at 4 $\mu\text{g l}^{-1}$ (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 $\text{Hg}^{2+} \text{l}^{-1}$ 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

*E-mail: ccantin@ifremer.fr

salinities for the early development of *Mytilus galloprovincialis* (His et al. 1989). All glassware was acid-washed (HNO₃ 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 log₂ 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, AC₅₀, and 50% mortality, LC₅₀, 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 H₀ = 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 AC₅₀ for embryos was 10 µg l⁻¹ and the LC₅₀ were 51, 164, 322 and 383 µg l⁻¹ for D-shaped, early umbonate, late umbonate and eyed larvae respectively. The ln LC₅₀ 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 (0.34,2.42) null	85.0 (79.11,90.36) null	97.6 (93.45,99.99) 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 (2.19,45.74) null	86.2 (72.81,99.21) 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 (1.99,41.13) reduced	19.9 (8.43,33.68) null	71.1 (66.61,74.55) null	99.0 (95.93,99.58) null

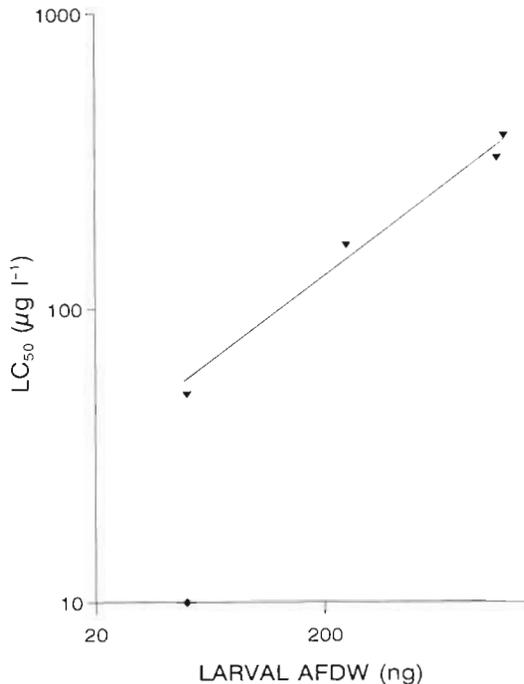


Fig. 1. *Mytilus galloprovincialis*. Double logarithmic plot relating Hg concentration causing 50% larval mortality after 48 h (LC_{50}) to larval ash-free dry weight (AFDW). (◆) Hg concentration causing 50% abnormalities in embryogenesis (AC_{50}) assuming no AFDW change during the endotrophic development

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 $\mu\text{g l}^{-1}$ from Day 6, and at Hg concentrations $> 1 \mu\text{g l}^{-1}$ 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 $\mu\text{g Hg l}^{-1}$. This prevented us from fitting straight lines. Rather, growth retardation was further investigated relating average growth rate (GR , $\mu\text{m d}^{-1}$) to Hg concentration (C , $\mu\text{g l}^{-1}$). The following highly significant regression model was obtained (20°C, 35 ppt):

$$GR = 11.9 (\pm 0.50) - 0.80 (\pm 0.121) C,$$

$$r = -0.967, p = 0.007.$$

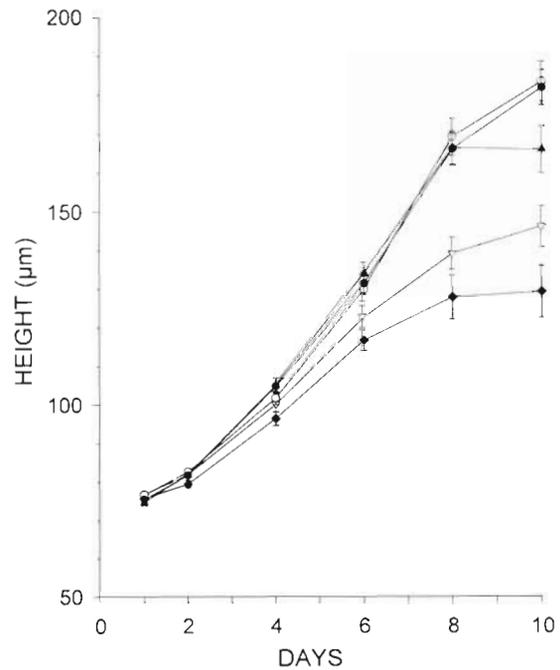


Fig. 2. *Mytilus galloprovincialis*. Height increase in larvae reared from D-shaped stage at 0 (●), 1 (○), 2 (Δ), 4 (▲) and 8 (◆) $\mu\text{g Hg l}^{-1}$ over 10 d. Error bars: \pm SE

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 ($\mu\text{g Hg l}^{-1}$) 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 $\mu\text{g Hg l}^{-1}$ 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 $b = 0.60 \pm 0.049$ (\pm 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.

Acknowledgements. We thank Dr M. Albentosa for her help in dealing with the statistics computer software and D. Morgans for her kind editing assistance. R.B. was funded by a European Community contract (ERBCHBICT941295) during the course of the study.

LITERATURE CITED

- Beiras R, His E (1994) Effects of dissolved mercury on embryogenesis, survival, growth and metamorphosis of *Crassostrea gigas* oyster larvae. Mar Ecol Prog Ser 113:95–103
- Beiras R, His E (1995) Toxicity of fresh and freeze-dried hydrocarbon polluted sediments to *Crassostrea gigas* embryos. Mar Pollut Bull 30:47–49
- Beiras R, Pérez Camacho A (1994) Influence of food concentration on the physiological energetics and growth of *Ostrea edulis* larvae. Mar Biol 120:427–435
- Brereton A, Lord H, Thornton I, Webb JS (1973) Effect of zinc on growth and development of larvae of the Pacific oyster *Crassostrea gigas*. Mar Biol 19:96–101
- Butler R, Chapman PM, van den Hurk P, Roddie B, Thain JE (1992) A comparison of North American and West European oyster embryo-larval toxicity tests on North Sea sediments. Mar Ecol Prog Ser 91:245–251
- Calabrese A, Collier RS, Nelson DA, MacInnes JR (1973) The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Mar Biol 18:162–166
- Calabrese A, MacInnes JR, Nelson DA, Miller JE (1977) Survival and growth of bivalve larvae under heavy-metal stress. Mar Biol 41:179–184
- Calabrese A, Nelson DA (1974) Inhibition of embryonic development of the hard clam, *Mercenaria mercenaria*, by heavy metals. Bull environ Contam Toxicol 11(1):92–97
- Chapman PM, Morgañ JD (1983) Sediment bioassays with oyster larvae. Bull environ Contam Toxicol 31:438–444
- Cossa D, Thibaud Y, Roméo M, Gnassia-Barelli M (1990) Le mercure en milieu marin, biogéochimie et écotoxicologie. Rapports scientifiques et techniques de l'IFREMER. No. 19. IFREMER, Brest
- His E, Robert R (1981) Effects of copper chloride on the eggs and D-larvae of *Crassostrea gigas* (Thunberg). Preliminary results. Comm Meet int Counc Explor Sea C.M.-ICES/F.43
- His E, Robert R (1982) Le danger des traitements par le sulfate de cuivre en zone conchylicole: toxicité vis-à-vis des oeufs et des jeunes larves de *Crassostrea gigas*. Revue Trav Inst (scient tech) Pêch marit 45:117–125
- His E, Robert R, Dinét A (1989) Combined effects of temperature and salinity on fed and starved larvae of the Mediterranean mussel *Mytilus galloprovincialis* and the Japanese oyster *Crassostrea gigas*. Mar Biol 100:455–463
- Manugistics (1993). Statgraphics reference manual. Manugistics Inc, Rockville, MD
- Martin M, Osborn KE, Billig P, Glickstein N (1981) Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. Mar Pollut Bull 12: 305–308
- Okubo K, Okubo T (1962) Study on the bio-assay method for the evaluation of water pollution. II. Use of the fertilized eggs of sea urchins and bivalves. Bull Tokai reg Fish Res Lab 32:131–140
- Roberts MH (1987) Acute toxicity of tributyltin chloride to embryos and larvae of two bivalve mollusks, *Crassostrea virginica* and *Mercenaria mercenaria*. Bull environ Contam Toxicol 39:1012–1019
- Thain JE (1984) Effects of mercury on the prosobranch mol-

lusc *Crepidula fornicata*: acute lethal toxicity and effects on growth and reproduction of chronic exposure. Mar environ Res 12:285-309
Watling HR (1982) Comparative study of the effects of zinc,

cadmium and copper on the larval growth of three oyster species. Bull environ Contam Toxicol 28:195-201
Zar JH (1974) Biostatistical analysis. Prentice-Hall, Inc, Englewood Cliffs, NJ

This article was submitted to the editor

Manuscript first received: February 16, 1995
Revised version accepted: May 2, 1995