Ecotoxicology (2012) 21:1617–1624
DOI 10.1007/s10646-012-0936-x

Toxic effects of benzo[*a*]pyrene (Bap) and Aroclor1254 on embryogenesis, larval growth, survival and metamorphosis of the bivalve *Meretrix meretrix*

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Accepted: 24 May 2012 / Published online: 9 June 2012

Abstract To assess the potential toxicity of polycyclic aromatic hydrocarbons and polychlorinated biphenyls on the early development of *Meretrix meretrix*, the effects of benzo[*a*]pyrene (Bap) and Aroclor1254 on embryogenesis and larval development were investigated using static laboratory toxicity tests at nominal concentrations of 6.25–1,600 μg/L. Even at 1,600 μg/L, Bap and Aroclor1254 only caused minor reductions in embryo development rates. The 96 h LC_{50} values for D-shaped larvae were 156 μg/L for Bap and 132 μg/L for Aroclor1254, respectively. The most sensitive toxicity endpoint in this study was metamorphosis, with an EC_{50} value of 20 μg/L for Bap and 35 μg/L for Aroclor1254. Aroclor1254 was more toxic than Bap to embryos and larvae. Our results indicate that Bap and Aroclor1254 do not show extreme toxicity to *M. meretrix* embryos and larvae. These data provide information for evaluating the toxicity of Bap and Aroclor1254 on bivalve embryos, especially over the entire larval stages.

Keywords Benzo[*a*]pyrene · Aroclor1254 · Embryogenesis · Larvae · Metamorphosis · *Meretrix meretrix*

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are common pollutants in marine environments. At present, global discharges of PAHs into the aquatic environment from all sources, both natural and anthropogenic, have been estimated at more than 80,000 tons per year (Wright and Welbourn 2002). PAHs are known to be toxic, genotoxic and carcinogenic, and to bioaccumulate (Lyons et al. 2002; Pérez-Cadahía et al. 2004; Siu et al. 2004). Benzo[*a*]pyrene (Bap), a carcinogen/teratogen that is included in the 16 PAHs designated by United States Environmental Protection Agency as priority contaminants, is highly toxic to aquatic organisms (Akcha et al. 2000) and has been widely investigated in ecotoxicology (Martínez and Livingstone 1995).

Polychlorinated biphenyls (PCBs) are among the most persistent organic pollutants and give great concern for marine ecosystems (Stebbing et al. 1992). PCB concentrations in marine environments, especially in coastal areas with rapid development, are still not decreasing because of continual input into the environment mainly from leakage from landfills and emissions from incinerators (Tanabe 1988). They bioaccumulate very easily and are highly toxic to aquatic organisms (Porte and Albaigés 1994; Niimi 1996; Magnusson et al. 2006). Aroclor1254, one of the typical commercial PCBs, has been used in toxicology research (Cheung et al. 2004; Iwanowicz et al. 2005).

In marine environments, bivalves have been used as models in the field of environmental toxicology (Rittschof and McClellan-Green 2005). In fact, the early developmental stages are often the most sensitive in the life cycle of bivalves. These stages are highly sensitive to toxicants such as pesticides (Wessel et al. 2007), antifouling paints (Bellas 2006) and heavy metals (His et al. 1999). Embryos and larvae of bivalves are can also be used to test the

toxicity of pollutants (Beiras and His 1994). Embryos and larvae of *Crassostrea* and *Mytilus* have already been proposed as organisms in marine ecotoxicological testing for assessing seawater quality (His et al. 1997a).

The clam *Meretrix meretrix*, widely distributed along the coastal and estuarine areas of East Asia, has become one of the most important ecological and commercial marine bivalves in China (Wang et al. 1993). It mainly inhabits the lower intertidal and shallow subtidal areas, which are particularly susceptible to be impacted by PAHs (from coastal oil spills) and PCBs. These coastal areas provide critical spawning and rearing habitats for many marine bivalve species. Therefore, the embryos and larvae may suffer from acute toxicity from accident oil leaks or chronic low-level organic contamination. Its larvae have been shown to be highly sensitive to heavy metals (Wang et al. 2009) and can be used as a bioassay for testing the toxicity of pollutants.

There have been only a few studies focusing on the toxicity of PAHs to bivalve embryos and larvae (Pelletier et al. 1997; Geffard et al. 2002; Lyons et al. 2002; Jeong and Cho 2005; Bellas et al. 2008). However, efforts have mainly focused on embryo toxicity, and the effects of PAHs on larval growth and mortality have been much less studied. Moreover, there are few reports about the toxicity of Aroclor1254 to bivalve embryos and larvae. The present study was conducted to determine the effects of Bap and Aroclor1254 on the embryogenesis and larval development of *M. meretrix*. This research may provide preliminary data for evaluating the effects of these pollutants on population recruitment in this species.

Materials and methods

Experimental solutions and analysis

Because of their low solubility in seawater, Bap and Aroclor1254 (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in dimethylsulfoxide (DMSO, GR, Guoyao, Shanghai, China) to prepare a stock solution at 2.5 g/L. This stock solution was diluted with filtered seawater to achieve the experimental concentrations. The maximal concentration of DMSO was 640 μg/L, which was found not to be toxic to embryos and bivalves in preliminary tests. Six concentrations, 0 (control), 6.25, 25, 100, 400 and 1600 μg/L were assayed.

Concentrations of Bap and Aroclor1254 in the experimental solutions were verified by gas chromatography–mass spectroscopy (Agilent 7890A, Agilent Technologies, Inc., Wilmington, DE, USA). Fifty milliliters of the experimental solution was extracted with dichloromethane three times and the extract was concentrated in a rotary evaporator to 1.0 mL. Then the solutions were analyzed according to the method described by Liu et al. (2006).

Brood stock and larva collection

Adults of *M. meretrix* were collected from Wenzhou (Zhejiang province, P. R. China) and reared at ambient temperature (28 ± 1 °C) in aquaria with filtered seawater. To induce spawning, the adults were taken out of the seawater and placed in the shade for 5–6 h, then transferred to tanks and stimulated using flowing seawater. Gametes were released after 2 or 3 h allowing fertilization. The zygotes then developed into the larval stage at an optimal temperature (28 °C). The D-shaped larvae were collected using a 50-μm mesh and cultured in 2 L tanks at an initial density of 4–6 individuals per mL.

Embryotoxicity experiments

The zygote develops to the D-shaped larva about 24 h after fertilization at the optimal temperature of 28 °C. Thus, the embryotoxicity experiments lasted for 24 h. For this assay, two-cell embryos (taken approximately 15 min after fertilization) were exposed to Bap or Aroclor1254 at the five different concentrations in beakers containing 100 mL seawater (three replicates per treatment). The number of embryos in each beaker was calculated before adding Bap or Aroclor1254. The density of the embryos was about 6–10 per mL, which had no effect on embryo development.

After 24 h, the larvae were fixed with formalin and the number of normal D-shaped larvae per beaker was recorded using microscopy. The endpoint measured was the percentage of normal D-shaped larvae, excluding embryos and abnormally developed larvae. Larvae were considered abnormal when they were an irregular shape, had a convex hinge, and/or protruding mantle (His et al. 1997a; Fig. 1). More than 150 embryos per treatment were observed to determine the embryogenesis rate. This experiment was repeated three times. The median effective concentration (EC_{50}) and 10 % effective concentration (EC_{10}) were defined as the concentrations that resulted in 50 and 10 % abnormal development, respectively.

Growth and survival experiments

These experiments were carried out on the D-shaped larval stage. Larvae (2–3/mL) were reared in 2 L polyethylene vessels and exposed to Bap or Aroclor1254 during larval development (three replicates per treatment). The larvae in all treatments were fed with *Isochrysis* spp. at a concentration of 1–10 × 10^4 cells/mL three times a day. The culture medium was renewed with fresh seawater daily. Optimal temperature (28 ± 1 °C), salinity (20 ‰) and pH (7.8) were controlled throughout the exposure and air was supplied by gentle aeration.

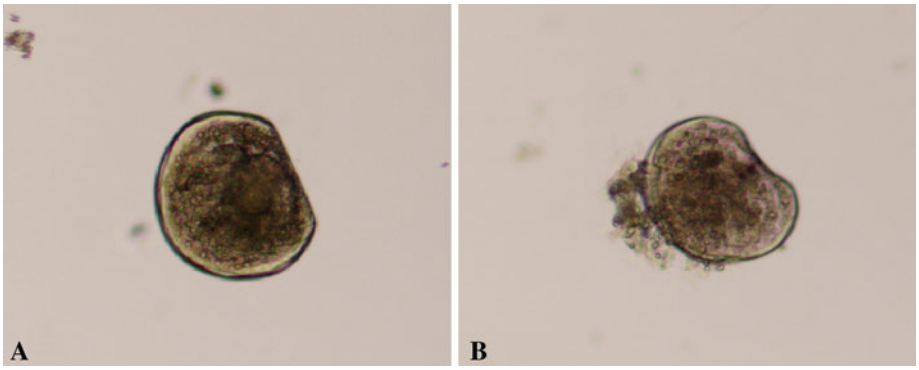

Fig. 1 Normal (**a**) and abnormal (**b**) D-shaped larvae of *M. meretrix* 24 h after fertilization

Because the shell lengths and heights of *M. meretrix* are correlated stably, shell length can be used as an appropriate indicator of growth (Tang et al. 2006). The mean length of the larvae (30 individuals per treatment) was recorded with a graduated eyepiece daily. *M. meretrix* only needs about 4–5 days to complete development from D-shaped larvae to the postlarval stage at 28 ± 1 °C. Thus, the duration of the exposure was 96 h, during which the larvae were at the pelagic stage.

The EC_{50} for growth was defined as the concentration that resulted in 50 % reduction in growth. The numbers of dead and live larvae (more than 50 individuals per treatment) were noted daily. The LC_{50} (median lethal concentration) was defined as the concentration that resulted in 50 % mortality.

Metamorphosis experiments

The experiment was carried out on D-shaped larvae exposed to Bap or Aroclor1254 at the stated concentrations. The larvae (2–3/mL) were reared in 2 L polyethylene vessels and fed with *Isochrysis* spp. at a concentration of 1–10 × 10^4 cells/mL three times a day. The culture medium was renewed with fresh seawater daily. Optimal temperature (28 ± 1 °C), salinity (20 ‰) and pH (7.8) were controlled throughout the exposure and air was supplied via gentle aeration. All the larvae in controls and treatments settled or died after 120 h exposure. Thus, the duration of this experiment was 120 h.

At the beginning of this experiment, the number of D-shaped larvae was calculated. At the end of the experiment, the number of postlarvae was recorded when all the larvae had settled completely. The EC_{50} for metamorphosis was defined as the concentration that resulted in 50 % reduction in settlement.

Statistical analysis

Values are presented as the mean ± SD. One-way analysis of variance (one-way ANOVA) was performed on all data using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA) and $P < 0.05$ was accepted as significant. Percentage data were transformed (arcsine of square root) before running the ANOVA and are presented in the figures as nontransformed percentages. EC_{50}, EC_{10}, and LC_{50} calculations were normalized to the control mean percentage of larval abnormality using Abbot's formula (Emmens 1948), $P = (Pe - Pc/100 - Pc) \times 100$, where Pc and Pe are control and experimental percentage responses, respectively. These values and their 95 % confidence intervals (CIs) in this experiment were calculated by the probit method (Newman 1995).

Results

Chemical analyses

The measured concentrations in this study are shown in Table 1. The concentrations of Aroclor1254 in the vessels ranged from 58 to 74 % of the nominal concentrations and the concentrations of Bap only account for 32–38 % of the nominal concentrations. Therefore, measured concentrations were used for calculation of toxicity parameters.

Table 1 Nominal versus measured concentrations (μg/L) for Bap and Aroclor1254 in test solutions used to determine the toxicity in developing embryos and larvae of *M. meretrix*

Nominal concentration	6.25	25	100	400	1600
Measured Bap	2.0 ± 0.2	8.3 ± 0.4	37 ± 5	153 ± 13	596 ± 43
Measured Aroclor1254	4.6 ± 0.7	17.5 ± 1.1	61 ± 7	230 ± 28	984 ± 73

Values are shown as mean ± SD (n = 3)

Effects of Bap and Aroclor1254 on embryogenesis of *M. meretrix*

When embryos were exposed to higher concentrations of Bap and Aroclor1254 (400, 1600 μg/L) for 24 h, the percentage of normal larvae showed a significant decrease ($P < 0.05$). Thus, Bap and Aroclor1254 inhibited embryo development in a dose-dependent manner when the concentration was ≥400 μg/L. The three lower concentrations of Bap and Aroclor1254 had no significant effects on embryogenesis (Fig. 2).

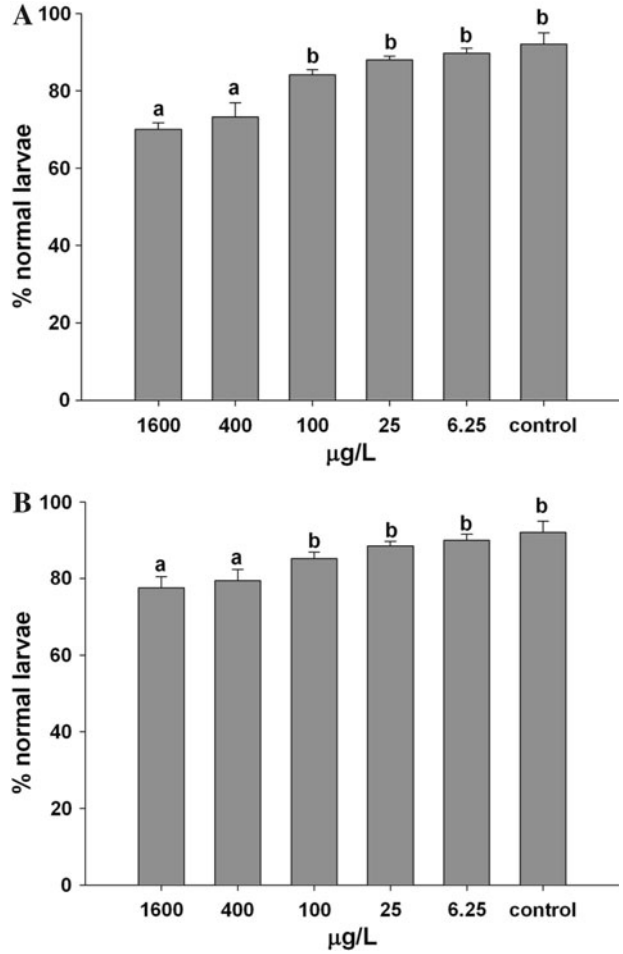

Fig. 2 Effects of Aroclor1254 (**a**) and Bap (**b**) on the percentage of *M. meretrix* embryos completing embryogenesis to form normal D-shaped larvae

The EC_{50} and EC_{10} values for embryo and their 95 % CIs are shown in Table 2. Because 1,600 μg/L Bap and Aroclor1254 only reduced the embryogenesis by a about 20 % with respect to the control, the EC_{50} values were above 596 μg/L for Bap and above 984 μg/L for Aroclor1254. The EC_{10} values for Bap and Aroclor1254 were 101 and 70 μg/L, respectively. According to the EC_{10} values, the toxicity of Bap was about 1.5 times higher than Aroclor1254.

Effect of Bap and Aroclor1254 on larval growth and survival of *M. meretrix*

The rates of shell length increase of *M. meretrix* exposed to Bap and Aroclor1254 are presented in Fig. 3. Concentration dependence in growth inhibition was found for all the concentration tested; 400 and 1,600 μg/L Bap significantly inhibited larval growth at all exposure times ($P < 0.05$), while 100 μg/L Bap had significant effects on growth only at 72 and 96 h. Doses of 100, 400 and 1600 μg/L Aroclor1254 significantly inhibited growth ($P < 0.05$). Larvae exposed to 400 and 1,600 μg/L Aroclor1254 barely grew at all, with shell length increases of 13 and 8 μm, respectively. However, the shell length of larvae in 1,600 μg/L Bap reached 174 μm, with a shell length increase of 41 μm. According to the EC_{50} values (Table 2), the toxicity of Aroclor1254 to larval growth was at least three times more than Bap.

These organic pollutants not only had severe effects on larval growth, but also had adverse affects on larval survival, as shown in Fig. 4. Concentration dependence in inhibiting survival was also evident. Doses of 6.25 and 25 μg/L Bap had no influence on larval mortality, whereas at 100, 400 and 1600 μg/L, Bap significantly inhibited larval survival ($P < 0.05$). Similar to Bap, doses of 100, 400 and 1600 μg/L Aroclor1254 significantly increased larval mortality ($P < 0.05$). Based on the LC_{50} values (Table 2), Bap was about 1.2 times more toxic than Aroclor1254.

Effect of Bap and Aroclor1254 on metamorphosis

As illustrated in Fig. 5, <10 % of larvae exposed to 100 μg/L Bap and Aroclor1254 settled and no postlarval stage was found at levels of 400 and 1,600 μg/L of either

Table 2 EC_{50} (EC_{10}, LC_{50}) values and their 95 % CIs of Bap and Aroclor1254 (μg/L) on embryogenesis, larval survival and metamorphosis of *M. meretrix*

	Embryogenesis EC_{50} (μg/L)	Embryogenesis EC_{10} (μg/L)	Growth EC_{50} (μg/L)	Larval mortality 96 h LC_{50} (μg/L)	Metamorphosis EC_{50} (μg/L)
Bap	>596	101 (0.4–317)	>596	156 (72–332)	20 (18–22)
Aroclor1254	>984	70 (8.7–178)	181 (134–241)	132 (61–240)	35 (32–38)

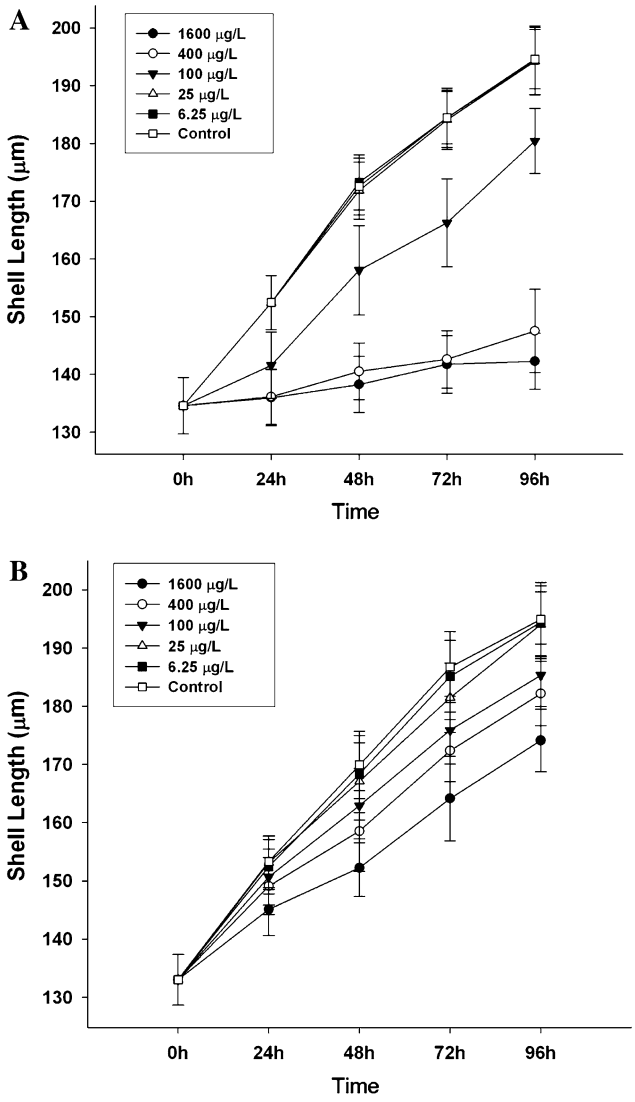

Fig. 3 Effects of Aroclor1254 (**a**) and Bap (**b**) on larval growth of *M. meretrix*

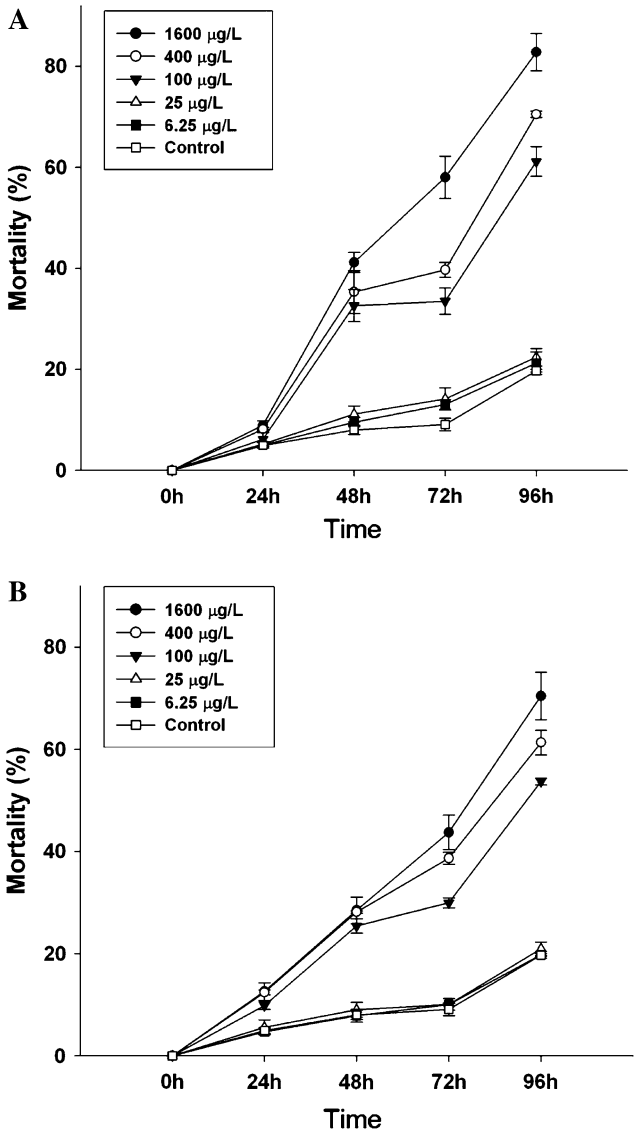

Fig. 4 Effects of Aroclor1254 (**a**) and Bap (**b**) on the mortality of *M. meretrix* larvae

agent. The numbers of postlarvae in the three higher concentrations (100, 400 and 1600 μg/L) of Bap and Aroclor1254 were significantly less than the numbers in control cultures ($P < 0.05$), while there were no differences between the two lower concentration and controls. The EC_{50} values for metamorphosis and their 95 % CIs are shown in Table 2. The toxicity of Bap was about 1.8 times greater than Aroclor1254.

Discussion

Effect of Bap and Aroclor1254 on embryogenesis

Studies on the toxicity of PAHs to marine invertebrate embryos have been carried out for several decades. Pelletier et al. (1997) studied the toxicity of anthracene, fluoranthene and pyrene on the bivalve *Mulinia lateralis* embryos and larvae. Oyster (*Crassostrea gigas*) embryos were found vulnerable to Bap exposure (Lyons et al. 2002; Jeong and Cho 2005; Wessel et al. 2007). Bellas et al. (2008) evaluated the toxicities of several PAHs with mussel, sea urchin and ascidian embryo/larval bioassays.

In this study, because the highest concentrations used did not cause a 50 % decrease in embryogenesis rates, the EC_{50} values were not calculable. Bap and Aroclor1254 showed very low toxicity to *M. meretrix* embryos in this study, although Aroclor1254 was a little more toxic than Bap. The toxicity of Bap to *M. meretrix* embryos was less than that for other bivalves. In *C. gigas*, 2.5 μg/L Bap significantly inhibited embryo development (Lyons et al. 2002). Jeong and Cho (2005) found that the percentage of

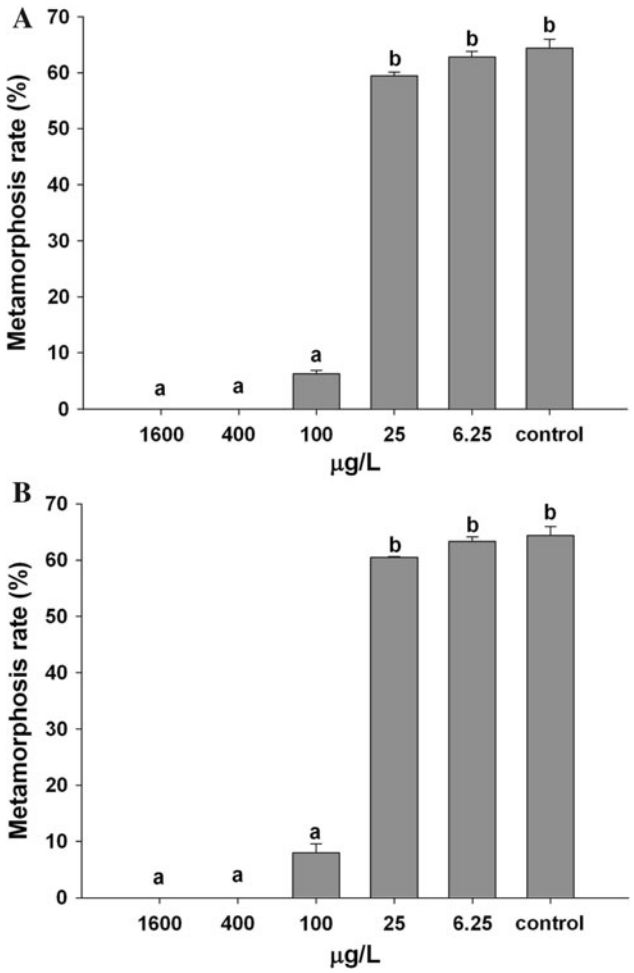

Fig. 5 Effects of Aroclor1254 (**a**) and Bap (**b**) on D-shaped larvae completing metamorphosis to postlarvae

normal D-shaped larvae of *C. gigas* developing from embryos exposed to 50–200 μg/L of a mixture of ten PAHs ranged from 57.1 to 68.3 %, compared with the control group (95.3–96.9 %). Wessel et al. (2007) demonstrated that 0.05–500 μg/L Bap showed significant embryotoxicity to *C. gigas*, however, there were still about 45 % embryos exposed to 500 μg/L Bap that developed to normal D-shaped larvae, compared with about 72 % in the control. These results indicate that embryos of different bivalve species show different sensitivities to Bap. However, Bap did not show acute toxicity to bivalve embryos. Weis and Weis (1982) found that eight batches of eggs exposed to concentrations of 0.01–10.0 mg/L Aroclor1254 had no effect on embryonic development or hatching of the fish *Fundulus heteroclitus*. These results suggested that Aroclor1254 has low toxicity to fish embryos.

The exposure period should be taken into account when comparing toxicities between experiments, because the duration of dosing is a major factor (Beiras and His 1994). Our exposure period was 24 h, which was shorter than the common exposure time of 48 h for tests on *C. gigas*. Moreover, the sensitivity of *M. meretrix* embryos might be lower than *C. gigas* embryos, as *M. meretrix* as an estuarine and intertidal species, might be more adaptive to environment changes than the oyster. These may explain the differences between our results and previous studies on the oyster.

Our study found that Bap and Aroclor1254 did not show high toxicity to *M. meretrix* embryos. In marine environments, the concentrations of Bap and Aroclor1254 are much lower than the values studied here. Thus, there is little chance of Bap and Aroclor1254 toxicity to *M. meretrix* embryos in most sea areas, unless there is massive oil leakage.

Effects of Bap and Aroclor1254 on larval growth and survival

Typically, toxicants first affect larval behavior (swimming) and physiological responses (growth), then lead to mortality. Thus, the larval growth test is often used as an endpoint in toxic bioassays. Although it is difficult to carry out, it is more sensitive and useful for a realistic assessment of the impact of a potential pollutant on wild fauna (Geffard et al. 2002). In our study, larval growth was not sensitive to Bap exposure, whereas it was more sensitive to Aroclor1254. Although the larvae exposed to 400 and 1,600 μg/L Bap could grow to about 170 μm, most suffered severe sublethal effects, such as injury to the velum and inhibition of swimming and feeding. In fact, these larvae may be associated with greater predation or find feeding more difficult in harsh natural environments.

The 96 h mortality is often used as an endpoint in acute toxicity tests. In the present study, the life cycle from D-shaped larvae to postlarvae was also about 96 h. Thus the 96 h toxicity measured the toxicity of Bap and Aroclor1254 to all larval stages except for the trochophore stage, a stage without feeding. Compared with larval growth, mortality was more sensitive to Bap and Aroclor1254 exposure. In fact, Bap and Aroclor1254 did not show extremely high toxicity to larvae compared with heavy metals. However, Farina et al. (2008) found that 10 μg/L Bap could significantly inhibit survivorship of *Porites astreoides* (coral) larvae after 48 h exposure. The coral larvae seemed to be more sensitive than *M. meretrix* larvae.

Our research suggests that the endpoint of growth and mortality was more sensitive than embryogenesis. Similar results have also been found in fish. Thus, Schimmel et al. (1974) reported that *Cyprinodon variegatus* larvae were more sensitive to Aroclor1254 than embryos. Nebeker et al. (1974) found that embryos of the fathead minnow and flagfish were more resistant to Aroclor1242 than the larvae

of these species. However, this result was different from those found from heavy metal toxicity testing (Beiras and His 1994; Geffard et al. 2002). The high toxicity of heavy metals to embryos may be ascribed to chemical interference with shell development, possibly calcium metabolism in the shell (Nice et al. 2000).

Effects of Bap and Aroclor1254 on larval metamorphosis

The EC_{50} values for metamorphosis were the lowest of the values, so this endpoint was the most sensitive in our research. This endpoint was more sensitive than the 96 h mortality, possibly because malformed larvae could still survive in the seawater and reach a shell length competent to settle, but could not finish metamorphosis. In addition, the duration of this endpoint was 24 h longer than 96 h mortality. In other invertebrates, larval metamorphosis was found to be more sensitive to PAH exposure. His et al. (1997b) found that elutriates of PAH-polluted sediment drastically reduced larval (pediveligers) metamorphosis of *C. gigas* after 48 h, but had no effect on larval mortality. Cebrian and Uriz (2007) reported that 1 μg/L PAH significantly reduced the percentage of settlement in the sponge *Crambe crambe* after 10 days. Bivalve populations mainly rely on larval settlement for maintenance. We demonstrated in this study that pollutant exposure could result in lower metamorphosis rates, which could cause long-term impacts on their population.

During these experiments, losses of Aroclor1254 and Bap might have occurred, primarily through adsorption and volatilization (Roesijadi et al. 1976; Pelletier et al. 1997). Therefore, the toxicities might have been underestimated in this study. In addition, photoactivation of PAHs at naturally relevant levels could increase the risk of Bap in the marine environment. The magnitude of increase in PAHs toxicity under ultraviolet light often exceeds a factor of 100 (Pelletier et al. 1997). Therefore, further study on the phototoxicity of Bap is necessary.

Conclusions

The impacts of Bap and Aroclor1254 exposure on the early life stages of *M. meretrix* were studied under laboratory conditions. Bap and Aroclor1254 did not show high toxicity to embryogenesis, larval survival and growth except for larval metamorphosis. Toxicity testing using embryos was less sensitive than the larval mortality and growth rates. The percentage of metamorphosed larvae exhibited a more sensitive response to Bap and Aroclor1254 and was more suitable for evaluating the toxicity to almost the entire larval stage. This study indicates that Bap and Aroclor1254 do not appear to pose a risk to *M. meretrix* embryos and larvae. In addition, these data provide biological criteria for the implementation of marine water quality standards.

Acknowledgments The authors thank for Dr. Zhihua Lin for the suggestions on this research and the help of staff in Qingjiang hatchery, Zhejiang Mariculture Research Institute. This research was supported by the Science Fund for Creative Research Groups (40821004), the National Key Foundational Research Project of China (no. 2007CB407305) and the National Marine Public Welfare Research Project (no. 200805069).

References

Akcha F, Izuel C, Venier P, Budzinski H, Burgeot T, Narbonne JF (2000) Enzymatic biomarker measurement and study of DNA adduct formation in benzo(*a*)pyrene-contaminated mussels, *Mytilus galloprovincialis*. Aquat Toxicol 49:269–287

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

Bellas J (2006) Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates. Sci Total Environ 367:573–585

Bellas J, Saco-Álvarez L, Nieto Ó, Beiras R (2008) Ecotoxicological evaluation of polycyclic aromatic hydrocarbons using marine invertebrate embryo-larval bioassays. Mar Pollut Bull 57:493–502

Cebrian E, Uriz MJ (2007) Contrasting effects of heavy metals and hydrocarbons on larval settlement and juvenile survival in sponges. Aquat Toxicol 81:137–143

Cheung CCC, Siu WHL, Richardson BJ, De Luca-Abbott SB, Lam PKS (2004) Antioxidant responses to benzo[*a*]pyrene and Aroclor 1254 exposure in the green-lipped mussel, *Perna viridis*. Environ Pollut 128:393–403

Emmens CW (1948) Principles of biological assay. Chapman and Hall, London

Farina O, Ramos R, Bastidas C, García E (2008) Biochemical responses of Cnidarian larvae to mercury and benzo(*a*)pyrene exposure. Bull Environ Contam Toxicol 81:553–557

Geffard O, Budizinski H, His E (2002) The effects of elutriates from PAH and heavy metal polluted sediments on *Crassostrea gigas* (Thunberg) embryogenesis larval growth and bio-accumulation by the larval of pollutants from sedimentary origin. Ecotoxicology 11:403–416

His E, Seaman MNL, Beiras R (1997a) A simplification the bivalve embryogenesis and larval development bioassay method for water quality assessment. Water Res 31:351–355

His E, Budzinski H, Geffard O, Beiras R (1997b) Effects of a hydrocarbon polluted sediment on *Crassostrea gigas* (Thunberg) metamorphosis. C R Acad Sci III 320:797–803

His E, Beiras R, Seaman MNL (1999) The assessment of marine pollution-bioassays with bivalve embryos and larvae. In: Southward AI, Tyler PA, Young CM (eds) Advances in marine biology, vol 37. Academic Press, London, pp 1–178

Iwanowicz LR, Lerner DT, Blazer VS, McCormick SD (2005) Aqueous exposure to Aroclor 1254 modulates the mitogenic response of Atlantic salmon anterior kidney T-cells: indications of short- and long-term immunomodulation. Aquat Toxicol 72:305–314

Jeong WG, Cho SM (2005) The effects of polycyclic aromatic hydrocarbon exposure on the fertilization and larval

development of the Pacific oyster, *Crassostrea gigas*. J Shellfish Res 24:209–213

Liu WX, Chen JL, Lin XM, Tao S (2006) Distribution and characteristics of organic micropollutants in surface sediments from Bohai Sea. Environ Pollut 140:4–8

Lyons BP, Pascoe CK, McFadzen IRB (2002) Phototoxicity of pyrene and benzo(*a*)pyrene to embryo-larval stages of the pacific oyster *Crassostrea gigas*. Mar Environ Res 54:627–631

Magnusson K, Ekelund R, Grabic R, Bergqvist PA (2006) Bioaccumulation of PCB congeners in marine benthic infauna. Mar Environ Res 61:379–395

Martínez PG, Livingstone DR (1995) Benzo[*a*]pyrene-dione-stimulated oxyradical production by microsomes of digestive gland of the common mussel, *Mytilus edulis* L. Mar Environ Res 39:185–189

Nebeker AV, Puglisi FA, Defoe DL (1974) Effect of polychlorinated biphenyl compounds on survival and reproduction of the fathead minnow and flagfish. Trans Am Fish Soc 103:562–568

Newman MC (1995) Quantitative methods in aquatic toxicology. Lewis Publishers/CRC Press, Boca Raton, p 426

Nice HE, Thorndyke MC, Morritt D, Steele S, Crane M (2000) Development of *Crassostrea gigas* larvae is affected by 4-nonylphenol. Mar Pollut Bull 40:491–496

Niimi AJ (1996) Evaluation of PCBs and PCDD/Fs retention by aquatic organisms. Sci Total Environ 192:123–150

Pelletier MC, Burgess RM, Ho KT, Kuhn A, McKinney RA, Ryba SA (1997) Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environ Toxicol Chem 16:2190–2199

Pérez-Cadahía B, Laffon B, Pásaro E, Méndez J (2004) Evaluation of PAH bioaccumulation and DNA damage in mussels (*Mytilus galloprovincialis*) exposed to spilled Prestige crude oil. Comp Biochem Physiol C 138:453–460

Porte C, Albaigés J (1994) Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustaceans, and fishes. Arch Environ Contam Toxicol 26:273–281

Rittschof D, McClellan-Green P (2005) Molluscs as multidisciplinary models in environment toxicology. Mar Pollut Bull 50:369–373

Roesijadi G, Petrocelli SR, Anderson JW, Giam CS, Neff GE (1976) Toxicity of polychlorinated biphenyls (Aroclor 1254) to adult, juvenile, and larval stages of the shrimp *Palaemonetes pugio*. Bull Environ Contam Toxicol 15:297–304

Schimmel SC, Hansen DJ, Forester J (1974) Effects of Aroclor 1254 on laboratory-reared embryos and fry of sheepshead minnows (*Cyprinodon variegatus*). Trans Am Fish Soc 103:582–586

Siu WHL, Cao J, Jack RW, Wu RSS, Richardson BJ, Xu L, Lama PKS (2004) Application of the comet and micronucleus assays to the detection of B[*a*]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). Aquat Toxicol 66:381–392

Stebbing ARD, Dethlefsen V, Carr M (1992) Biological effects of contaminants in the North Sea. Mar Ecol Prog Ser 91:1–361

Tanabe S (1988) PCB problems in the future: foresight from current knowledge. Environ Pollut 50:5–28

Tang BJ, Liu BZ, Wang GD, Zhang T, Xiang JH (2006) Effects of various algal diets and starvation on larval growth and survival of *Meretrix meretrix*. Aquaculture 254:526–533

Wang RC, Wang ZP, Zhang JZ (1993) Marine shellfish aquaculture. Qingdao Ocean University Press, Qingdao, pp 322–324 (in Chinese)

Wang Q, Liu BZ, Yang HS, Wang XY, Lin ZH (2009) Toxicity of lead, cadmium and mercury on embryogenesis, survival, growth and metamorphosis of *Meretrix meretrix* larvae. Ecotoxicology 18:829–837

Weis P, Weis JS (1982) Toxicity of the PCBs Aroclor 1254 and 1242 to embryos and larvae of the Mummichog, *Fundulus heteroclitus*. Bull Environ Contam Toxicol 28:298–304

Wessel N, Rousseau S, Caisey X, Quiniou F, Akcha F (2007) Investigating the relationship between embryotoxic and genotoxic effects of benzo[*a*]pyrene, 17-α-ethinylestradiol and endosulfan on *Crassostrea gigas* embryos. Aquat Toxicol 85:133–142

Wright DA, Welbourn P (2002) Environmental toxicology. Cambridge University Press, Cambridge