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Chronic Toxicity Thresholds for Sediment-Associated Benzo[a]pyrene in the Midge (*Chironomus dilutus*)

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Abstract Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in aquatic ecosystems and have been shown to be one of the causes of sediment toxicity to benthic invertebrates. Benzo[a]pyrene (BaP) was selected as a representative for the PAH family of compounds for developing chronic sediment toxicity thresholds for *Chironomus dilutus*. Life-cycle toxicity testing was initiated using newly hatched midge larvae and terminated until hatch of the second generation. Median lethal concentrations were 92.5 ± 19.6 and 56.9 ± 1.76 μg/g organic carbon (OC) after exposing midges to sediment-associated BaP for 20 days (before pupation) and 43 days (end of test), respectively. Sublethal toxicity was described as 5 and 50 % effect concentrations (EC5 and EC50), and these were 6.63 ± 0.82 and 41.1 ± 1.61 μg/g OC for growth reduction at 20 days, respectively. Impairments of emergence and reproduction of *C. dilutus* were also assessed at the end of the testing, and the EC5 and EC50 values were 3.41 ± 0.53 and 26.9 ± 1.43 μg/g OC for emergence and 2.18 ± 0.34 and 13.4 ± 1.13 μg/g OC for reproduction, respectively. In addition, bioavailability-based chronic toxicity thresholds were also established using Tenax-extractable BaP concentrations. Although more environmentally relevant, data regarding chronic toxicity are less available than those regarding acute toxicity. Therefore, establishing numeric chronic toxicity thresholds for sediment-associated BaP with the consideration of the bioavailability would improve the accuracy of assessing PAH-related sediment toxicity.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in sediment and pose a threat to sediment-dwelling organisms. Due to their increased concentrations, PAHs have been shown to be the major contributors to acute toxicity to benthic organisms in some sediments (Mehler et al. 2010). Acute bioassays are useful for analyzing the hazards of highly toxic chemicals and contamination at high levels, but do not test key life-stage events, such as moulting and reproduction, during which organisms' susceptibility to toxicants may increase, and thus they do not show the long-term effects of contaminant exposure at low levels. Alternatively, chronic exposure to low levels of PAHs in sediment is more environmentally relevant (McCarty et al. 1992). Fewer data, however, are available regarding toxicity in benthic invertebrates after chronic exposure to PAHs in sediment. It is desirable to develop chronic toxicity thresholds as benchmarks for assessing the risk of PAH-contaminated sediment.

As hydrophobic organic contaminants, PAHs are generally associated with organic carbon (OC) in sediment, and thus the benchmarks derived from equilibrium partitioning theory have been proposed to estimate the toxicity

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of PAHs to sediment-dwelling organisms (United States Environmental Protection Agency 2003). In addition to OC content, OC type also strongly affects the bioavailability of PAHs in sediment. For example, condensed OC, such as black carbon, coal, and kerogen, is more capable to sorb planar PAHs than amorphous OC (Cornelissen et al. 2005). To improve the accuracy of sediment-risk assessment, the bioavailability of sediment-associated PAHs should also be considered when determining toxicity thresholds.

Because of their ecological importance, wide geographical distribution, and ease of culturing, *Chironomus dilutus* (Diptera, Chironomidae, with an alias *C. tentans*) has been recommended by the USEPA for sediment toxicity testing in freshwater ecosystems (United States Environmental Protection Agency 2000). The life cycle of *C. dilutus* consists of four larval stages, a pupal stage, and a nonfeeding adult stage, and it normally takes 3–4 weeks to complete a whole life cycle (Gower and Buckland 1978). The midges live in sediment during all larval stages. This benthic invertebrate, during its larval stage, burrows in sediment and almost never leaves, feeding mainly on detrital particles around the tunnel it makes. Although the pupa might sometimes swim to the top of the sediment, it soon emerges as the nonfeeding adult.

The objectives of the present study were as follows: (1) to evaluate the adverse effects of the representative PAH, benzo[a]pyrene (BaP), on the survival of *C. dilutus* during a whole life-cycle exposure test, starting from newly hatched larvae (<24 h old), as well as sublethal effects, including decreases in growth, emergence, and reproduction; and (2) to establish numerical chronic toxicity thresholds for BaP to *C. dilutus* for better understanding of the relationship between BaP concentrations (OC-normalized sediment concentrations and 24-h Tenax-extractable concentrations) and its adverse effects, which were represented as the 5 % effect (EC5), median effect (EC50), and median lethal concentrations (LC50).

Materials and Methods

Chemicals and Reagents

Because its toxicity is relatively greater than that of other PAHs, BaP was selected as the representative for the PAH family of compounds. BaP was purchased from SpexCertiprep Inc. (Metu, NJ, USA) with certified purity of >97 %. Perylene-d12 (Thermo Fisher Scientific, Waltham, MA, USA) was added to all of the samples before extraction and used as the surrogate to verify the performance of analytical processes. Before chemical analysis on gas chromatography-mass spectrometry (GC-MS), *p*-terphenyl (Dr. Ehrenstorfer GmbH, Augsburg, Germany) was added to the extracts as the internal standard for GC-MS quantification.

Hexane (high-performance liquid chromatography grade) was purchased from Burdick & Jackson (Ulsan, Korea). Dichloromethane and acetone (analytical grade) were obtained from Tianjin Chemical Reagent Factory (Tianjin, China) and redistilled before use. The drying agent anhydrous Na_2SO_4 was purchased from Tianjin Chemical Reagent Factory and baked at 450 °C for 4 h before being used to remove residual water from the extracts. Before use, neutral silica gel (80–200 mesh) and alumina (80–200 mesh) were activated at 180 and 250 °C for 12 h, respectively. Tenax TA sorbents (60–80 mesh) were purchased from Scientific Instrument Service, Inc. (Ringoes, NJ, USA). Reconstituted water was prepared by dissolving various salts in high-purity deionized water according to USEPA protocol and used as the overlying water in the bioassays (United States Environmental Protection Agency 2000). The water contained $NaHCO_3$, $MgSO_4$, KCl, $CaCl_2$, and $CaSO_4$ at concentrations of 96, 30, 4, 50, and 50 mg/l, respectively, and was aerated for at least 24 h before use (United States Environmental Protection Agency 2000).

Sediment Spiking

Control sediment was collected from a drinking water reservoir in Conghua, China. Sediment samples were sieved through a 2-mm sieve on site and transported to Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIGCAS), where they were sieved through a 500-μm sieve, homogenized, and stored at 4 °C. Previous toxicity testing and analytical work showed that the control sediment had limited contamination and did not exhibit adverse effects to benthic organisms (Du et al. 2013). After inorganic carbon was removed by 1 mol/l HCl solution, total OC content of the sediment was analyzed using an Elementar Vario EL III elemental analyzer (Hanau, Germany) with a value of 1.60 ± 0.14 %. Before conducting toxicity testing, preliminary chronic toxicity tests were performed with sediments spiked with BaP at a wide range of concentrations. Referring to the estimated EC50 values of BaP in preliminary testing, appropriate amounts of BaP were added to the sediment using acetone as carrier. The spiked sediments were then thoroughly mixed for 4 h using a drill with a rotating stainless-steel blade. The spiked sediments were stored at 4 °C for 17 days and homogenized again before initiation of the bioassays. Sediment concentrations of BaP are listed in Table 1.

Bioassays

Chronic exposure of *C. dilutus* to BaP-contaminated sediment was performed using 400-ml beakers containing 60 g of wet sediment, and 250 ml of overlying reconstituted water was used for each replicate. The midges were cultured at GIGCAS in accordance with USEPA protocols, and newly hatched larvae (<24 h old) were used for the

Table 1 Concentrations of BaP in sediment analyzed at time 0 (beginning of testing), at 20 days (before pupation), and at 43 days (end of whole life-stage toxicity testing)

Treatment	Concentration (μg/g OC)			
	0 day	20 days	43 days	Average
C1	14.4 ± 0.66	10.3 ± 3.64	14.8 ± 0.89	13.2 ± 2.87
C2	122 ± 10.0	111 ± 30.0	117 ± 7.34	116 ± 16.9
C3	138 ± 20.9	120 ± 15.1	126 ± 9.71	128 ± 15.9
C4	193 ± 21.7	188 ± 26.2	168 ± 2.79	183 ± 20.6
C5	290 ± 29.0	291 ± 19.1	288 ± 4.06	290 ± 17.5
C6	356 ± 26.9	328 ± 14.3	308 ± 8.66	331 ± 26.5
C7	569 ± 11.5	534 ± 37.2	514 ± 7.11	539 ± 31.1
C8	667 ± 30.0	677 ± 35.8	627 ± 14.2	657 ± 33.4

Data are presented as means ± SDs of three replicates. Average sediment concentrations of three time points are also shown ($n = 9$)

Table 2 Lethal and sublethal end points assessed in the bioassays

Toxicity		End point	Observation group[a]
Lethal	Mortality	Mortality at 20 days (before pupation)	A
		Mortality at 43 days (end of testing)	B
Sublethal	Growth	AFDM/replicate at 20 days	A
	Emergence	Cumulative emergence	B
		Rate of emergence	B
	Reproduction	Number of females per replicate	B
		Number of eggs per female	B
		Total number of eggs per replicate	B
		Egg hatchability	B

[a] Observation group means end points data were determined by observing the midges in the group

bioassays (United States Environmental Protection Agency 2000). Twenty newly hatched midge larvae were placed into each beaker after the sediment was allowed to settle overnight. The testing was performed under a 16:8-h light-to-dark photoperiod, and water temperature was held at 23 ± 1 °C with temperature, pH, conductivity, and dissolved oxygen monitored daily and ammonia measured weekly. The overlying water was renewed twice daily with 100 ml of reconstituted water each time. Considering their physiological differences at the different life stages, midge larvae were fed once every day with 1 ml of different concentrations of grounded fish food: no feeding during the first 2 days, 0.6 g/l during the next 5 days, 3 g/l for the other 5 days, and 6 g/l until the end of the testing. No BaP was detected in fish food.

The organisms in the tests were divided into three groups (labeled A, B, and C), with which different toxic end points were evaluated. Three replicates were used for each group. Specific end points used are listed in Table 2, and the evaluation of toxic end points followed the methods reported in our previous study (Du et al. 2013).

Group A

Lethality and growth impairments were chosen as the end points for group A. At the end of 20-day exposure, midges from group A were sieved from the sediment, and mortalities were counted. Immobile midges, when pinched with a pair of forceps, were considered dead. In addition, growth of the midges was also determined by ash free dry mass (AFDM) measurements according to the method presented by Maul et al. (2008). Briefly, the surviving organisms were placed in preweighed aluminum pans and dried at 60 °C for 3 days to obtain mean mass per organism. Next, the organisms and pans were heated at 550 °C for 3 h and reweighed on a Sartorius AgPro 11 microbalance (Gottingen, Germany) to calculate the AFDM.

Group B

Both lethal (survival) and sublethal end points (impairments of emergence and reproduction) were measured for midges in group B. Similar to the process mentioned previously, mortality of midges from group B was counted at the end of the life-cycle tests. Whole life-cycle toxicity testing was terminated at 43 days, which was 7 days after emergence of the last adult midge of the controls. Sublethal end points were obtained by recording the changes of midges in group B throughout the whole life-cycle exposure.

Group C

Midges in group C were used to supply auxiliary males for the female adults that emerged from group B.

Chemical Analysis

BaP was extracted from sediment samples using a CW-2000 ultrasound-assisted microwave extractor (UAME)

(Xintuo Company, Shanghai, China) (Du et al. 2012). Approximately 10 g of freeze-dried sediment was extracted with 100 ml of a hexane and acetone (1:1, v/v) solution for 360 s using UAME after adding the surrogates. Activated copper powder was added to the sediment to remove sulfur. The UAME power of ultrasound and microwave was set at 50 and 100 W, respectively. The extracts were then filtered and evaporated and the solvent exchanged to approximately 1 ml of hexane using a XT-NS-1 Turbovap (Xintuo Company). Columns with an internal diameter of 1 cm were packed with 12 cm of silica gel, 6 cm of alumina, and 1 cm of anhydrous Na_2SO_4 from bottom to top and used to clean the extracts, and 70 ml of a 30 % dichloromethane in hexane solution was used as the eluting solution. The cleaned extracts were then concentrated and solvent exchanged to appropriate volumes of hexane. After the addition of the internal standard of *p*-terphenyl, the extracts were analyzed on GC-MS.

Analysis of BaP was performed using an Agilent 7890-5975 GC-MS (Santa Clara, CA, USA) with electron impact ionization in selected ion monitoring mode after separation with an Agilent HP-5 MS column (30 m × 0.25 mm i.d. × 0.25-μm film thicknesses). Helium was used as carrier gas at a flow rate of 1.2 ml/min. The injection was performed in splitless mode at 280 °C with an injection volume of 1 μl. Temperature of transfer line, ion source, and quadrupole was set at 260, 230, and 150 °C, respectively. Column temperature was ramped from 80 to 230 °C at 20 °C/min, held for 1 min, and finally ramped to 290 °C at 15 °C/min and held for 7.5 min. Identification of BaP and the surrogate (perylene-d12) was completed by detecting the target and qualifier ions within a retention time window of 1 %, and the target ion for BaP had an *m/z* of 252. Five-point internal standard calibration curves were used to quantify the analytes.

A calibration standard was analyzed after every ten samples on GC-MS, and relative differences between the calibration curve and the daily calibrations were within 20 % for all analytes. A method blank (solvent), matrix blank (control sediment), matrix spike, and matrix spike duplicate were included in sediment analysis for every 20 samples. No target compounds were detected in the blanks. In addition, the surrogate perylene-d12 was added to all samples before sediment extraction to evaluate the efficiency of the sample preparation process, and the average recovery of perylene-d12 was 114 ± 21.8 %.

In addition, single-point Tenax extraction at 24 h was used to estimate the bioavailability of BaP in sediment according to a previously developed method (Mehler et al. 2011a). Briefly, after the spiked sediments were aged for 17 days, approximately 5 g of wet sediment, 0.5 g of Tenax, 5 mg of NaN_3, which was used to prevent microbial growth, and 45 ml of reconstituted water were added into a 50-ml tube. The tubes were rotated at 20 revolutions/min for 24 h on a QB-228 rolling incubator (Kylin-Bell Laboratory Instruments Co., China). On completion, Tenax beads were separated from the sediment slurry and sonicated with 5 ml of acetone and 5 ml of a mixture of acetone and hexane (1:1, v/v) twice sequentially. The sonication time was 5 min for each extraction. After the surrogate was added, the extracts were combined and concentrated and the solvent exchanged to 1 ml of hexane, cleaned with the alumina/silica gel columns mentioned previously, and then concentrated and solvent-exchanged to hexane. After addition of the internal standard, BaP in the extracts were assessed on GC-MS.

Data Analysis

Growth, emergence, reproduction, and survival data from the bioassays were used to determine EC5, EC50, and LC50 values using Probit analyses with SPSS 13.0 software (IBM, Chicago, IL, USA). The effect data of growth, emergence, and reproduction (ECx-G, ECx-E, and ECx-R, respectively) were all normalized to the controls by dividing the adverse effects in the BaP-spiked treatments by the respective responses in the controls before estimating the EC values; control mortality was included for LC50 estimation. All toxicity thresholds were expressed using two types of dose metrics, i.e., the OC normalized bulk sediment concentrations and the Tenax-extractable sediment concentrations. Toxicity among the treatments was compared using analysis of variance and Dunnett's multiple comparison test using SPSS 13.0 software. Significant difference was set at p value <0.05.

Results and Discussion

Sediment samples were analyzed at the beginning, at 20 days, and at the end of the bioassays. Concentrations of BaP in control sediments were lower than the detection limit. As listed in Table 1, no significant difference was noted for BaP concentrations throughout the course of the bioassays; thus, average sediment concentrations were used. In all bioassays, dissolved oxygen (5.4 ± 1.1 mg/l), pH (7.73 ± 0.12), temperature (23.3 ± 0.4 °C), conductivity (342 ± 11 μs/cm), and ammonia (0.35 ± 0.12 mg/l) of the overlying water were monitored, and all of the water parameters were within acceptable ranges as required by the United States Environmental Protection Agency (2000). Lethality and sublethal impairments of growth, emergence, and reproduction were investigated by exposing *C. dilutus* to BaP-spiked sediments for a whole life cycle.

Lethality

Lethality of *C. dilutus* was influenced by the presence of BaP in sediment. As shown in Fig. 1 and Supplementary

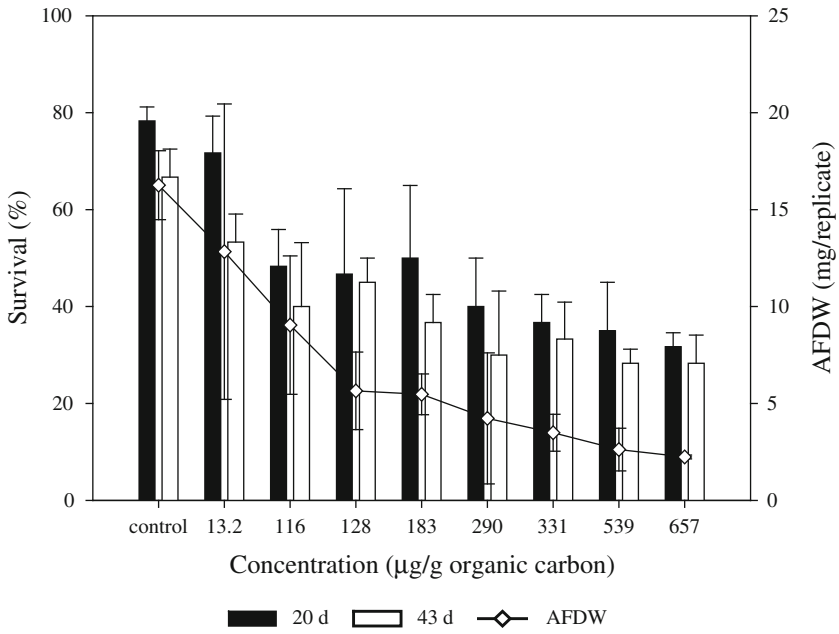

Fig. 1 Percent survival and AFDM (mg/replicate) of *C. dilutus* after chronic exposure to various concentrations of BaP in sediment. Survival was assessed at 20 days and at the end of life-cycle toxicity testing (43 days). Growth, presented as AFDM of midge larvae, was only measured at 20 days

Table S1, the toxicity of BaP to midges was evident with LC50 values of 92.5 ± 19.6 and 56.9 ± 1.76 µg/g OC after 20- and 43-day exposures, respectively. Information on the chronic toxicity of BaP is sparse, and most studies were focused on the acute toxicity of low-ring PAHs (Suedel et al. 1993; Verrhiest et al. 2001). Verrhiest et al. (2001) found in 10-day acute toxicity tests that phenanthrene and fluoranthene caused 50 % mortality of *C. riparius* at concentrations of 20 and 15 µg/g dw, respectively. The same magnitudes of the 10-day LC50 values (3 to 8.7 µg/g dw) were also reported for *C. riparius* exposed to fluoranthene (Suedel et al. 1993). In addition to the potent of different PAHs, the divergence of LC50 values might be related to different organism stages and lengths of exposure periods used in testing: We used 24-h newly hatched midge larvae and a whole life cycle, whereas the other investigators used third-instar midges plus a 10-day exposure (Suedel et al. 1993; Verrhiest et al. 2001). Earlier, MacDonald et al. (2000) reported that in freshwater ecosystems, the probable effect concentration of BaP was 76 µg/g OC, and BaP concentrations inducing the lowest and highest apparent effects were 160 and 360 µg/g OC, respectively. Although no specific aquatic organisms were mentioned in the study by MacDonald et al. (2000), lethality data of BaP in our study for *C. dilutus*, one of the most frequently used test organisms in freshwater bioassays (United States Environmental Protection Agency 2000), were close to those effect concentrations.

The results of the present study started with newly hatched larvae, which were more vulnerable to contamination. Therefore, achieving high survival of larvae in the controls is extremely important to make sure the results are valid and reliable. Survivorship of *C. dilutus* after a 43-day exposure is shown in Fig. 1. Results showed that survival rates of the midges from control sediments all exceeded the minimum of those required by the United States Environmental Protection Agency (2000), which were no <70 % at 20 days and 65 % at the end of test. With BaP concentrations increased, midge survival rates decreased from 78.3 ± 2.9 and 66.7 ± 5.8 % in the controls to 31.7 ± 2.9 and 28.3 ± 5.8 % in the highest BaP-spiked sediment at 20 and 43 days, respectively. In our previous work (Du et al. 2012), survival of 94 ± 6.5 % was observed for midges exposed to the same control sediment. The difference between the two studies was the small size, increased frailty, and sensitivity of the newly hatched larvae used in the present study.

At the end of the tests (43 days), average survival of the larvae in control sediments was approximately 14.8 % lower than that determined at 20 days, and similar phenomena have also been reported in two other studies using long-term life-cycle tests (Sibley et al. 1996, 1997). The loss of midges between 20 days and the onset of pupation of the fourth-instar larvae was the reason for the increased mortality (Benoit et al. 1997). In the process of transforming fourth-instar larva to pupa, mobilization of lipids in the organisms may liberate the contaminants, which were previously stored in the lipids, to the target sites. As a consequence, midge mortality increased.

Sublethal Toxicity

In addition to lethality, the adverse effect on growth was also measured using the AFDM of the midges after 20-day

exposure. When exposed to contaminants, midges must allocate more energy to maintain survival, thus leaving less energy for their growth (Jager et al. 2004). As shown in Fig. 1 and Table S1, the AFDM of *C. dilutus* generally decreased with increasing BaP concentrations, which were 16.3 ± 1.78 mg/replicate in control sediment and 2.20 ± 0.10 mg/replicate at the highest concentration of BaP. The AFDM of individual larva in each treatment was significantly lower than that in the control sediment; however, no significant differences among the treatments themselves were noticed. Together, these data suggested obvious sublethal toxicity of BaP to *C. dilutus* growth with EC5 and EC50 values of 6.63 ± 0.82 and 41.1 ± 1.61 μg/g OC, respectively.

Compared with the controls, a significant decrease in emergence was also noted after midges were exposed to BaP-contaminated sediment (Fig. 2). Emergence has been used in numerous studies with Chironomidae as a measurement end point for sublethal toxicity (Wentsel et al. 1977; Williams et al. 1986; Hatakeyama 1987; Pascoe et al. 1989), and the control emergence in this study was typical for control sediments in the life-cycle test (Sibley et al. 1996, 1997). Total emergence of *C. dilutus* exposed to the highest concentration of BaP was approximately 62.5 % lower than that in the controls (Fig. 2). The EC5 value for the total emergence of *C. dilutus* was 26.9 ± 1.43 μg/g OC. In addition to the total number of emerged adults, the rate at which emergence occurred also decreased with increasing treatment levels (Fig. 2). A similar phenomenon was also reported by Sibley et al. (1996) in their zinc toxicity study. Assessing the rate at which emergence occurs may be more informative than relying entirely on total emergence, especially in situations of low-level contamination (Sibley et al. 1997). A plot of cumulative emergence is particularly insightful because it provides information on both total emergence and rate of emergence (Benoit et al. 1997). As suggested in Fig. 2, the delay was significant in all BaP-spiked treatments for cumulative emergence compared with controls.

Developmental time for the midges could also be assessed using cumulative emergence, and it was consistently longer for midges exposed to BaP compared with the controls. For example, the developmental time required for 30 % of midges to be emerged from the control sediment was approximately 31 days compared with 33 days for the treatment at the lowest BaP concentration. The delay of midge development due to BaP exposure might be related to numerous biochemical and physiological changes that resulted in the failure of midge larvae to complete pupation, and thus fewer adults were emerged at greater concentrations levels (Taylor et al. 1993).

As shown in Fig. 3a, although no significant differences were observed in the number of eggs per female midge among the treatments, the number was slightly lower when

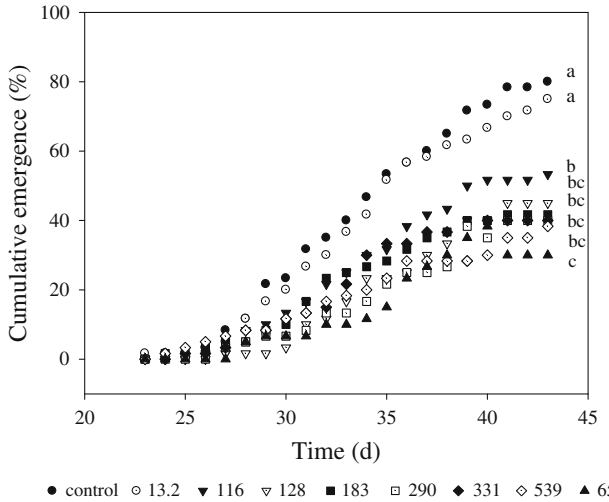

Fig. 2 Cumulative emergence of *C. dilutus* after exposure to various concentrations of BaP versus exposure time. Cumulative emergence at 43 days, when the last emergence was recorded, is presented with SD in the small graphs. Significant differences ($p < 0.05$) among the treatments are indicated by *different letters*

comparing BaP-spiked sediments with controls. Interestingly, egg production in controls was approximately 2–55 % lower than that in BaP-spiked treatments (except at the lowest level), and similar results have been previously reported (Benoit et al. 1997). Compared with BaP-spiked treatments, more organisms survived in the controls resulting in greater organism density. From various organism densities, we can infer that nutritional differences between different sediments might be one of the reasons why lower reproductive output occurred in controls. Determining the average number of the total eggs per treatment may also be informative, especially when the total number of eggs per treatment changed dramatically although no significant difference was observed among the average number of eggs per female (Benoit et al. 1997). Although not so obvious, a slight downward trend was observed in the total number of eggs per treatment when BaP concentrations increased with the exception of that in the highest BaP level. Specifically, the fecundity of *C. dilutus* was inhibited when exposed to low concentrations of BaP, but it was enhanced at high BaP concentrations. The average number of total eggs per treatment ranged from 1,720 ± 1,363 to 6,475 ± 5,247 in the BaP-contaminated sediments, whereas it was 3,893 ± 2,813 in the control sediment (Fig. 3b).

Adverse effects of prenatal BaP exposure on fetal development have been much investigated, and inhalation exposure of pregnant rats or mice of 25–100 μg/l BaP decreased the numbers of live pups at birth (Ramesh et al. 2002; Wu et al. 2003). Furthermore, injection of BaP to

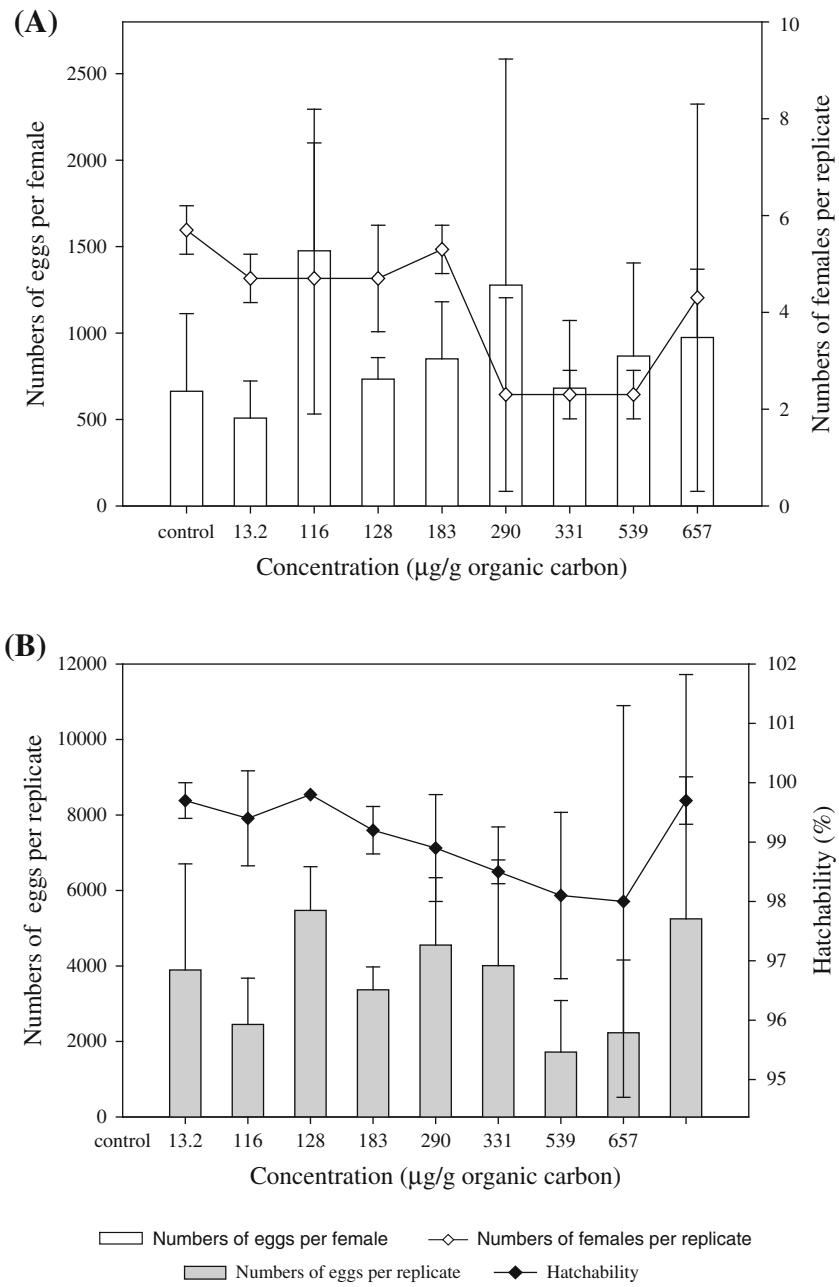

Fig. 3 Adverse effects of BaP on reproduction of *C. dilutus* with multiple end points, including number of females per treatment and number of eggs per female (**a**) as well as total number of eggs per treatment and hatchability of eggs (**b**)

pregnant mice also caused measurable changes in enzymes, i.e., pyruvate kinase and lactic acid dehydrogenase, in the lungs of the fetuses (Rady et al. 1981). Therefore, embryo viability (hatch rate of eggs or hatchability) was used as a toxic end point as well. As shown in Fig. 3b, a slight decrease in hatchability was observed with increasing BaP concentrations. However, just as Sibley et al. (1996) found in their study, no significant differences appeared among the treatments. An average of 99.1 % eggs were hatched for all treatments indicating that hatchability was not a sensitive end point for sediment toxicity assessment with the midges.

Chronic Toxicity Thresholds

Overall, chronic toxicity thresholds with OC-normalized sediment concentrations as the dose metrics were established by exposing *C. dilutus* to BaP-contaminated sediments for the whole life cycle (Fig. 4a). Although the no observed-effect concentration and the lowest observed effect concentration were conventionally used to express chronic toxicity, the reliability of the two indices came under debate recently (Jager 2012). Hence, in the present study, the marginal and toxic levels of chronic toxicity were expressed by the EC5 and EC50, respectively.

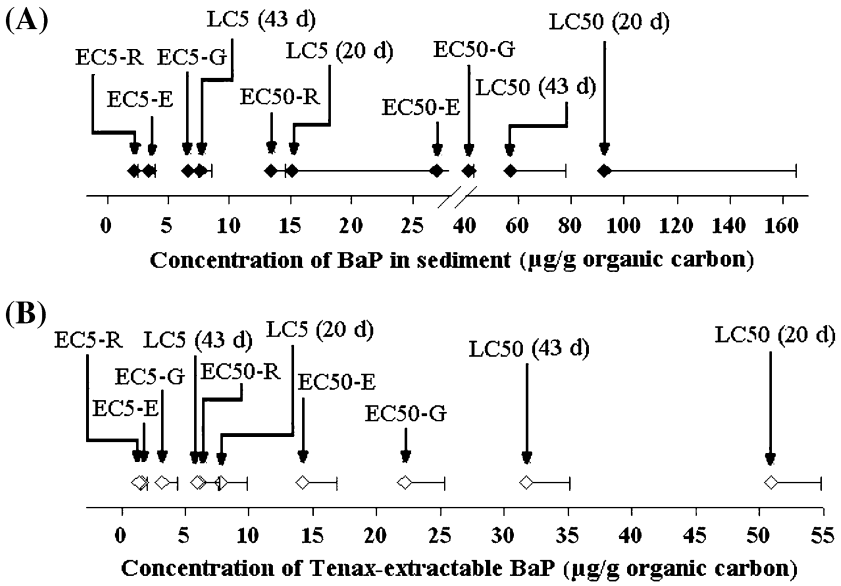

Fig. 4 Chronic toxicity thresholds with end points, including lethality and impairments of growth, emergence, and reproduction, of *C. dilutus* exposed to sediment-associated BaP. OC-normalized sediment concentration (**a**) and 24-h Tenax-extractable concentration (**b**) were used as dose metrics. *Solid* and *hollow diamonds* represent OC-normalized and Tenax extractable concentrations, respectively. The LC5 and LC50 represent 5 % and median lethal concentrations, and the EC5 and EC50 represent 5 % and median effect concentrations, respectively. The letters *R*, *E*, and *G* stand for reproduction, emergence, and growth, respectively. Data are presented as means plus SDs

As shown in Fig. 4a, BaP caused 50 % lethality in *C. dilutus* at 92.5 ± 19.6 and 56.9 ± 1.76 µg/g OC at 20 days (before pupation) and 43 days (the end of the testing), respectively, with EC50 values for growth, emergence, and reproduction of 41.1 ± 1.61, 26.9 ± 1.43, and 13.4 ± 1.13 µg/g OC, respectively. Reproduction was the most sensitive toxic end point with an EC5 of 2.18 ± 0.34 µg/g OC.

The development of chronic toxicity thresholds for sediment-associated BaP provides valuable benchmarks for evaluating ecological risk of this contaminant, particularly in aquatic ecosystems where the organisms are exposed to low levels of PAHs and exhibit no acute toxicity. For example, concentrations of BaP in sediments from the Pearl River Delta in China ranged from 0.111 to 18.3 µg/g OC (Mehler et al. 2011b), suggesting possible chronic sublethal toxicity in some sites. The decrease of their growth and emergence could negatively affect the population of the midges (Maul et al. 2008). Thus, the application of chronic toxicity thresholds for predicting long-term sublethal effects may also provide a better understanding of the impact of PAHs in sediment at the population level.

In addition, it is well known that the presence of black carbon in sediment significantly decreased the bioavailability of planar PAHs (Cornelissen et al. 2005). As a result, bulk sediment concentration of BaP overquantified its toxicity, and PAH body residues were proposed as dose metrics to incorporate bioavailability into toxicity evaluation (Landrum et al. 2003 However, to measure BaP concentration in midges is an analytical challenge because midges have relatively high biotransformation capacity and small body sizes (Gerould et al. 1983). Instead, inclusion of bioavailability measurements into toxicity thresholds could also increase accuracy in assessing sediment risk. Tenax extraction has been shown to be an effective method for estimating the bioavailability of hydrophobic organic contaminants in sediment (You et al. 2011). Therefore, additional set of chronic toxicity thresholds was also established using Tenax-extractable BaP concentrations as the dose metrics (Fig. 4b). As shown in Fig. 4, only a portion of BaP in sediment (the fraction of 24-h Tenax-extractable BaP concentration in bulk sediment concentration was 0.63 ± 0.09) was bioavailable to the midges, and the use of bioavailability-included dose metrics significantly decreased variations in toxicity thresholds.

Conclusion

Chronic toxicity thresholds were established by exposing *C. dilutus* to sediment-associated BaP for the whole life cycle, and toxic responses included mortality and multiple sublethal end points (impairments of growth, emergence, and reproduction). Both OC-normalized sediment and Tenax-extractable concentrations were used as dose metrics to incorporate the measurements of bioavailability and chronic

toxicity into hazard assessment. The development of chronic toxic thresholds for BaP provides valuable benchmarks to evaluate the ecological risk of PAHs in sediment.

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