PBDES ALTERS SPECIES-SPECIFIC LARVAL SETTLEMENT PATTERN OF MARINE BENTHIC POLYCHAETES AT ENVIRONMENTALLY RELEVANT CONCENTRATION

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Introduction

Polybrominated diphenyl ethers (PBDEs), which are extensively used as flame retardants, are found ubiquitously in marine environments worldwide. Sediment is the major sink of PBDEs, with the congener BDE 47 being most abundant[1]. Since larval settlement of marine invertebrates is dependent upon chemical characteristics of sediment in sediment surface[2], PBDEs may alter sediment characteristics, and hence larval settlement. However, the potential impact of PBDEs on larval settlement of marine benthic polychaetes remains largely unknown, albeit such information is clearly important in assessing the risk of PBDEs on the marine environment. In this study, laboratory experiments were carried out to test the hypothesis that immediate (i.e. 24 h) and prolonged (i.e. 4 weeks) contamination of BDE 47 in marine sediment can alter polychaete larval settlement. Using a multiple-choice experiment, larval settlement of three polychaete species (*Pseudopolydora vexillosa*, *Polydora cornuta*, and *Capitella* sp. I) on the following four types of sediment were studied and compared: (i) sediments spiked with low concentration of BDE 47 (0.5 ng g^{-1} dry weight); (ii) sediment spiked with high concentration of PBDE (3.0 ng g^{-1} dry weight), (iii) sediment spiked with acetone (solvent control), and (iv) untreated natural sediment (control).

Materials and Methods

Materials:

PBDEs and sediment collection: 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) with 99 % purity (GC-MS) was purchased from Wellington Laboratories, USA and was dissolved in acetone. Natural sediment was collected from Tung Lung Chau, east of Victoria harbor, Hong Kong (22°19´N, 114°16´E) using a van Veen grab (0.1 m^2). This site was selected because it is relatively clean and has low levels of pollutants[3,4]. Sediment collected was sieved through a 250 µm mesh, rinsed with natural seawater (NSW) and stored at -80 ℃ (designated as natural sediment). Sediment was frozen at -80^{o}C for 2 weeks to kill off the benthic animals/larvae therein, and thawed for 6 hours at 4 ℃ before being used for experiment.

Polychaete cultures: Culture techniques of *P. cornuta*, *P. vexillosa* and *Capitella* sp. I were adopted from elsewhere with minor modifications[3,5,6]. Adult worms were seeded in small culture containers with fully aerated seawater under a photoperiod of 12h light:12h dark at constant temperature (24 ℃) and salinity (32 ‰). Polychaete larvae were collected and rinsed into culture vessels with a variety of microalgae for cultivation. Competent larvae were defined and used for experiment according to

morphology and number of chaetigers[7] and setigers[8].

Methods:

Sediment preparation: Natural sediment was spiked with low (0.5 ng g^{-1} dry weight) or high (3.0 ng g^{-1} dry weight) concentrations of BDE 47. Sediment with and without acetone served as solvent control and control, respectively. All spiked sediments were vigorously vortexed after BDE 47 addition and kept at 4 ℃ overnight to prevent degradation. All sediment samples were centrifuged at 2,000 rpm for 5 min to remove excessive seawater prior to multiple choice settlement assays.

Multiple choice settlement assays: A replicated 4 x 4 Latin-square design was used for the larval choice experiment. A chamber, each with 4 × 4 wells (Ø 2 cm; depth 0.3 cm) milled out of plexiglass within a chamber (Ø 17 cm, height 1.5 cm), were set up for the multiple-choice experiment[10]. Four different types of sediment: (a) natural sediment (control), (b) sediment spiked with acetone (solvent controls), (c) sediment with high concentration of BDE 47, and (d) sediment with low concentration of BDE 47 were assigned to the wells following a Latin-square design with four replicates per treatment/control. Competent larvae of each polychaete species were added and the chamber was kept in darkness at room temperature. Total number of settled larvae/ juveniles in each well was counted after 24 h and 4 weeks, respectively. Each experiment was repeated trice (n = 3) for each species using larvae from different batches of larval cultures.

Quantification of PBDEs in sediment samples: Concentration of BDE 47 was measured before and after the multiple-choice settlement assays, following the procedure described in 11. Sediment samples were freeze-dried, grounded with anhydrous sodium sulfate (Sigma, USA) and Soxhlet extracted with a mixture of dichloromethane: hexane (3:1) for 8 h. Activated copper was added to desulphurize the samples, and eluent of BDE 47 was concentrated before passing through a column containing 4.0 g activated silica gel (Sigma 391484, USA) for further cleanup. $^{13}C_{12}$-labeled BDE 77 (Wellington Laboratories, USA) was added as a recovery spike prior to gas chromatograph (GC) analysis. BDE 47 in the sample was quantified using a GC (Agilent 7890) equipped with a mass-selective detector (Agilent 5975) with DB-5HT fused silica capillaries (J & W Scientific Inc.; 0.25 mm i.d. × 30 m × 0.10 µm film thickness).

Results and Discussion

Total numbers of larvae settled in different treatments and controls in the multiple choice settlement assays after 24 h and 4 weeks are shown in Figures 1 and 2 respectively. Immediate exposure of high concentration of BDE 47 (3.0 ng g^{-1} dry weight) in sediment increased larval settlement of *P. vexillosa* and *Capitella* sp. I by more than one-fold compared with that in the controls; whilst larval settlement of *P. cornuta* was not affected irrespective of BDE 47 concentration. Prolonged exposure of high BDE 47 concentration decreased and increased juvenile settlement of *P. cornuta* and *Capitella* respectively compared to the controls; whilst juvenile settlement was not affected by BDE 47. High concentration of BDE 47 impaired the growth of settled juveniles of *P. cronuta* and *Capitella* sp. I, respectively (Figure 3), which were highly correlated to the settlement results under long-term exposure. For the first time, our results demonstrated that short- and long-term exposure of BDE 47 at environmentally relevant concentration in marine sediment can alter larval/ juvenile settlement pattern of marine polychaetes in species-specific and concentration-dependent manners. Results will be presented/

discussed at the workshop.

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References

1. Lema SC, Schultz IR, Scholz NL, Incardona JP, Swanson P. 2007. Aquat Toxicol 82:296
2. Woodin SA. 1991. Am Zool 31:797
3. Thiyagarajan V, Soo L, Qian PY. 2005. J Exp Mar Biol Ecol 323:70
4. Thiyagarajan V, Soo L, Shin PKS, Qian PY. 2006. Mar Ecol Prog Ser 327:207
5. Mok F, Thiyagarajan V, Qian PY. 2008. J Exp Mar Biol Ecol 357:99
6. Irvine SQ, Martindale MQ. 1999. Mar Models Electron Rec 29
7. Hsieh HL. 1999. Invertebr Reprod Dev 25:205
8. Sebesvari Z, Esser F, Harder T. 2006. J Exp Mar Biol Ecol 337:109
9. Liu Y, Zheng GJ, Yu H, Martin M, Richardson BJ, Lam MHW, Lam PKS. 2005. 50:1173
10. Grassle JP, Butman CA, Mills SW. 1992. J Mar Res 50:717
11. Uneo D, Kajiwara N, Tanaka H, Subramaniam A, Fillmann G, Lam PKS, Zheng GJ, Muchitar M, Razak H, Prudente M, Chung KH, Tanabe S. 2004. Environ Sci Technol 38:2312