EFFECTS OF MARINE POPs ON THE EARLY LIFE DEVELOPMENT AND METAMORPHOSIS OF ECHINODERMS

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1. Introduction

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), and personal care products (PCPs) are widely present in the marine environment and tend to accumulate in the sediment and food web reaching concentrations that potentially cause toxic effects[1,2,3].

Early life stages (ELS) of vertebrates are regarded as the most sensitive to toxic effects of POPs[4,5]. Furthermore, early life development of several vertebrate species (*e.g.* amphibians) comprises a thyroid hormone (TH) dependent metamorphosis which is known to be sensitive to thyroid disrupting compounds (TDCs)[6].

Echinoderms live in close contact with sediment, where POPs tend to accumulate, thus making them particularly at risk for toxic effects[7,8]. Several studies have shown that the ELS of echinoderms are the most sensitive life stages to the toxic effects of POPs[9,10]. Similarly to amphibians and flatfish, echinoids (Echinodermata: Echinoidea) have a metamorphosis regulated by THs[11], consequently they may also be potentially susceptible to the effects of thyroid disrupting compounds (TDCs).

In the present study *Psammechinus miliaris* was used for the development of an extended echinoid ELS and a metamorphosis bioassay to effectively evaluate the potential adverse effects posed by POPs during early life development and metamorphosis. The development of echinoids as an animal model for (marine) ecotoxicological studies will also help addressing the ethical need to reduce the number of vertebrates currently used.

2. Materials and methods

2.1. ELS bioassay

P. miliaris embryos and larvae originating from own aquaculture were exposed to PCB 126 (3,3',4,4'5-pentachlorobiphenyl), TCS (Triclosan) and HBCD (hexabromocyclododecane technical mixture) from 0 to 16 days post fertilization (dpf). Twice a week 50 % of the exposure volume was removed and replaced with new sea water (SW) spiked at the respective test concentration. Toxic effects were quantified by sampling larvae at 1, 6, and 13 dpf into a 24 wells-plate (Fig. 1). In the 3 subsequent days to each sampling larvae were scored for developmental stage, morphological abnormalities and mortality.

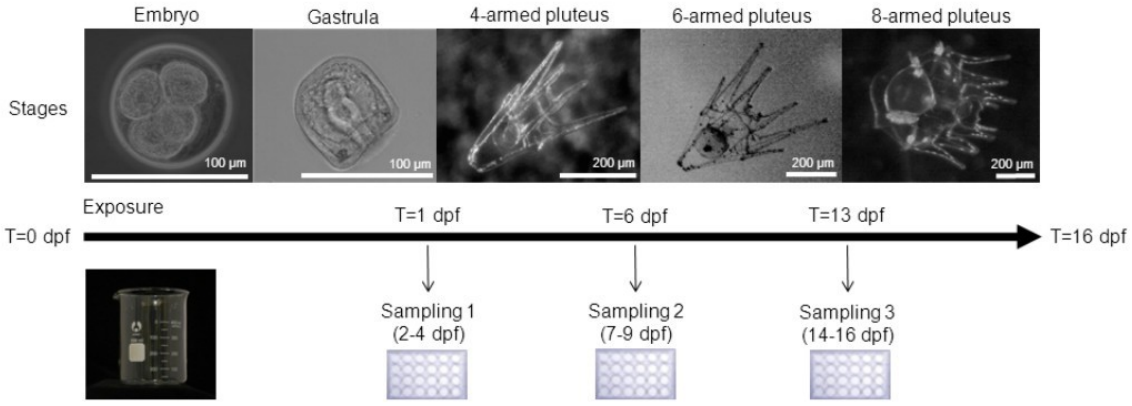

Figure 1. *P. miliaris* ELS bioassay experimental design.

Toxic effects on larval development rate were presented as "penalty points" following the same principle as introduced for quantification of tadpole development[12]. At 16 dpf larvae that develop normally are at the 8-armed pluteus stage and were accredited 0 penalty points. Larvae at an earlier development stage were accredited penalty points depending on the developmental stages lacking: 1 point when they were at the 6-armed pluteus stage, 2 points at the 4-armed pluteus stage, and 3 points for all earlier development stages.

2.2. Metamorphosis bioassay

P. miliaris larvae were reared in artificial sea water (ASW) until they reached the middle 8-armed pluteus stage with rudiment between stage J and K[11] (Fig 2.B). From 20 dpf onwards larvae were exposed to TBBPA (tetrabromobisphenol A) or a PBDE mixture (BDE-28, -47, -99, 100, 153/4) in 100 ml glass beakers in duplicate (Fig. 2). Twice a week 10 ml of ASW spiked at the desired test concentrations was added, once the volume in test beakers reached 80 ml half was removed and water refreshment continued as described.

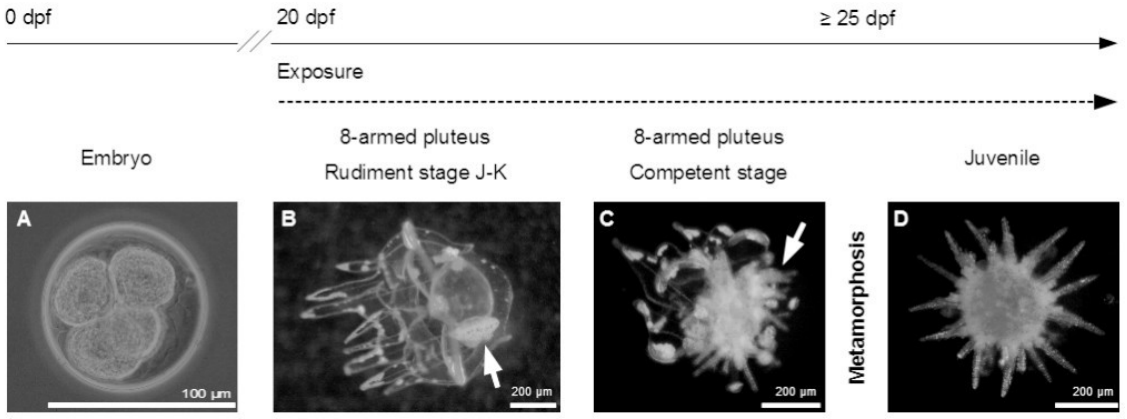

Figure 2. *P. miliaris* metamorphosis bioassay experimental design. Arrow indicates the larva rudiment. Toxic effects were quantified for the following end points: time to completion of metamorphosis, morphological abnormalities in juveniles, and mortality. Metamorphosis was considered completed when all the larvae body was reabsorbed and the test was terminated when 80% of the larvae in the control groups had completed metamorphosis.

3. Results and discussion

3.1. ELS bioassay

In contrast to results reported for amphibians and fish[13,14], *P. miliaris* early life development was not sensitive to dioxin-like toxicity at concentrations as high as 0.03 nM TCDD-equivalents (TEQs). The unexpected absence of dioxin-like toxicity points out the need to further investigate the mechanisms of dioxin-like toxicity in echinoids to avoid an underestimation of toxic effects that hamper their use as ecotoxicological animal models for this class of compounds.

TCS, on the other hand, was acutely toxic during embryo development at concentrations ≥ 500 nM, this acute effect is to be expected since this compound is commonly used for its biocide properties. Due to the extensive use in PCPs TCS is one of the most commonly detected chemicals in waste water in the USA[15]. Therefore, this compound presents a potential risk for aquatic organisms and a more complete assessment of its risks is particularly necessary to evaluate sub-lethal effects.

Early life development of *P. miliaris* was rather sensitive to HBCD with a remarkably steep dose-response curve. The effects included an increase in morphological abnormalities induced at test concentrations above 50 nM HBCD. The developmental penalty points measured at 16 dpf was the most sensitive endpoint to HBCD exposure with a NOAEL of 25 nM. This indicates that delayed development is an important sub-lethal effect that cannot be determined in common echinoid ELS tests since the observation period ends between 48 and 72h post fertilization.

The newly developed 16 days echinoid ELS bioassay proved to be sensitive to toxic effects of POPs (*i. e.* TCS and HBCD). The obtained results suggest that an extended observation period for echinoid bioassays is feasible and recommendable to avoid an underestimation of sub-lethal effects during early life development as we observed for HBCD exposure.

3.2. Metamorphosis bioassay

The PBDE mixture tested was selected based on the profile of PBDE congeners measured in sole (*Solea solea*) collected from the Western Scheldt estuary (The Netherlands). *P. miliaris* larvae exposed to the PBDE mixture showed a clear acceleration of metamorphosis at concentrations ≥32 nM (EC_{50} 219 nM). This finding suggests that the mixture acts as a TH partial agonists. PBDEs are structurally similar to THs, and their ability to interfere with TH function has been reported. However, *Xenopus laevis* tadpoles exposed to DE-71 (technical mixture) showed a delay in the time to completion of metamorphosis[6].

Exposure to TBBPA inhibited *P. miliaris* metamorphosis in a dose dependent manner (EC_{50} 97 nM) and concentrations ≥75 nM (EC_{50} 81 nM) increased the incidence of malformations in the few juveniles that were able to metamorphose. The observed malformations included severe edema and the absence of spines. TBBPA is also structurally similar to TH, and it has been classified as a possible agonist of TH action leading to accelerated amphibian metamorphosis[16]. As it was observed for the PBDE mixture, the antagonistic effects of TBBPA on *P. miliaris* metamorphosis are in contradiction with results reported for amphibians which could be explained by partial (ant)agonism.

Our results suggest that the mechanism by which THs regulate metamorphosis in echinoids might differ from vertebrates to a certain extent. In vertebrates triiodothyronine (T3) is regarded as the biologically active TH[17], while in echinoids thyroxin (T4) is suggested as the most potent TH regulating metamorphosis[11]. It is likely that other differences in TH function between vertebrates and echinoids also exist, including biotransformation potential, resulting in differences in the mechanism of TH disruption.

To our knowledge this study is the first evaluating the effects of POPs on the TH dependent metamorphosis of sea urchins. We provided evidence that TDCs (i. e. PBDEs and TBBPA) are able to disrupt TH function in echinoids in a dose dependent manner suggesting that echinoids can be a suitable ecotoxicological model to

study TH disruption. Future work investigating TH function in echinoids is recommended to understand the mechanisms of disruption compared to vertebrates.

4. Acknowledgements
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5. References

1. Fair, P.A., Lee, H.-B., Adams, J., Darling, C., Pacepavicius, G., Alaee, M., Bossart, G.D., Henry, N., Muir, D., (2009). Environ. Pollut. 157, 2248-2254.
2. Magnusson K, Ekelund R, Grabic R, Bergqvist PA, (2006); Mar. Environ. Res. 61: 379-395.
3. Moore MR, Vetter W, Gaus C, Shaw GR, Müller JF, (2002); Mar. Poll. Bull. 45: 62-68.
4. Hutchinson, T.H., Solbe, J., Kloepper-Sams, P.J., (1998). Chemosphere. 36, 129-142.
5. Van Leeuwen, C.J., Grootelaar, E.M.M., Niebeek, G., (1990). Ecotoxicol. Environ. Saf. 20, 42-52.
6. Balch, G.C., Vélez-Espino, L.A., Sweet, C. Alaee, M., Metcalfe, C.D., (2006). Chemosphere 64, 328-338.
7. Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P.E.G., van Leeuwen, S.P.J., de Boer, J., (2004). Environ. Sci. Technol. 38, 5497-5504.
8. Coteur, G., Gosselin, P., Wantier, P., Chambost-Manciet, Y., Danis, B., Pernet, P., Warnau, M., Dubois, P., (2003). Arch. Environ. Contam. Toxicol. 45, 190-202.
9. den Besten, P.J., Herwig, H.J., Zandee, D.I., Voogt, P.A., (1989). Ecotoxicol. Environ. Saf. 18, 173-180.
10. Pagano, G., Cipollaro, M., Corsale, G., Esposito, A., Ragucci, E., Giordano, G.G., Trieff, N.M., (1985). Mar. Environ. Res. 17, 240-244.
11. Chino, Y., Saito, M., Yamasu, K., Suyemitsu, T., Ishihara, K., (1994). Developmental Biology 161, 1-11.
12. Gutleb, A.C., Schriks, M., Mossink, L., Berg, J.H.J.v.d., Murk, A.J., (2007). Chemosphere. 70, 93-100.
13. Foekema, E.M., Deerenberg, C.M., Murk, A.J., (2008). Aquat. Toxicol. 90, 197-203.
14. Gutleb, A.C., Appelman, J., Bronkhorst, M.C., van den Berg, J.H.J., Spenkelink, A., Brouwer, A., Murk, A.J., (1999). Environ. Toxicol. Phar. 8, 1-14.
15. Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., (2002). Environm Sci Technol. 36, 227–232
16. Veldhoen, N., Boggs, A., Walzak, K., Helbing, C.C., (2006). Aquatic Toxicology, 78 (3), pp. 292-302.
17. Kistler, A., Yoshizato, K., Frieden, E., (1975). Endocrinology. 97, 1036-1042.