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Abnormalities in bivalve larvae from the Puck Bay (Gulf of Gdansk, southern Baltic Sea) as an indicator of environmental pollution

Rafal Lasota[a,*], Katarzyna Gierszewska[a], Frédérique Viard[b,c], Maciej Wolowicz[a], Katarzyna Dobrzyn[a], Thierry Comtet[b,c]

[a] University of Gdansk, Faculty of Oceanography and Geography, Department of Marine Ecosystems Functioning, Laboratory of Estuarine Ecology, Al. M. Pilsudskiego 46, 81-372 Gdynia, Poland

[b] Sorbonne Universités, UPMC Univ Paris 06, UMR 7144, Equipe DIVCO, Station Biologique, Roscoff, France

[c] CNRS, UMR 7144, Adaptation et Diversité en Milieu Marin, Station Biologique, Roscoff, France

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ABSTRACT

This study described the occurrence of abnormalities in bivalve larvae from the Puck Bay. Analyses of plankton samples collected in 2012–2013 showed that larval *Mytilus trossulus*, *Mya arenaria*, and *Cerastoderma glaucum* exhibited abnormalities that could indicate adverse environmental impacts. The deformities were mainly in shells, but missing soft tissue fragments and protruding vela were also noted. In addition to larval studies, we analyzed benthic postlarvae of *Mytilus trossulus*. Interestingly, grooves and notches at different locations of the prodissoconch, dissoconch, and shell margin were observed. Some of these deformations were reminiscent of the indentations found on the shell edge of larvae. Comparing the proportion of abnormal postlarvae to larvae with shell abnormalities suggested that the survival of larvae with shell abnormalities was low. Overall, our results suggested that the ratio of abnormal bivalve larvae could be used as an indicator of the biological effects of hazardous substances in the pelagic environment.

1. Introduction

Marine pollution affects coastal and estuarine ecosystems in many ways and at various scales, from the organism to community levels (Weis, 2014). Because of their sensitivity to pollutants and their bioaccumulation ability, marine invertebrates, especially mollusks, have long been used to monitor the biological effects of contaminants and environmental quality (Rainbow and Phillips, 1993) by measuring diverse molecular, cellular and physiological biomarkers (e.g., Bolognesi et al., 1996; Wedderburn et al., 2000; Leinio and Lehtonen, 2005). Early life stages (embryos and larvae) of marine invertebrates are more sensitive to pollutants than adults of the same species (Connor, 1972; Calabrese et al., 1973; Pineda et al., 2012), and, thus, they offer the possibility of detecting lower levels of pollution. Exposed embryos and larvae typically show retarded development, increased morphological abnormalities, and higher mortality (e.g., Calabrese et al., 1977; Klöckner et al., 1985; Fichet and Miramand, 1998; Beiras and Bellas, 2008; Mai et al., 2012; Stefansson et al., 2016). In the particular case of bivalves, slow growth, increased percentages of abnormal D-shaped veliger larvae, tissue necrosis resulting from damage to the shell gland, and abnormal velar structures are some of the documented effects of harmful compounds (Calabrese et al., 1977; His et al., 1999; Inoue et al., 2006). Such developmental alterations have been used to develop embryo-larval bio-assays, especially using oysters (*Crassostrea* spp.) and mussels (*Mytilus* spp.), to examine the effects of a variety of contaminants such as heavy metals, pesticides, detergents, and, ultimately, to assess contamination levels of waters and sediments (e.g., His et al., 1999; Wedderburn et al., 2000; Quiniou et al., 2007).

Bio-assays are typically carried out through embryo exposure to contaminants or to waters (or sediments) over a short period (24–48 h), with mortality and level of developmental abnormalities as the endpoints. Although abnormality rates in embryos and larvae are important in bio-assays and are recommended as one of the standard response variables to be measured routinely in assessments of multistressor effects (Przeslawski et al., 2015), they are targeted infrequently in field studies (but see Lehtonen et al., 2014 and references therein), especially in those of mollusks. One explanation for the scarcity of such approaches could be the difficulty of identifying larvae at the species level, especially in groups like bivalves. Not only are early larval stages very difficult to distinguish based only on morphology (Garland and Zimmer, 2002; Hendriks et al., 2005), morphological abnormalities caused by pollution can make identification even more difficult.

* Corresponding author.
E-mail address: ocerl@ug.edu.pl (R. Lasota).

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Advances in molecular-based methods of identifying species, i.e., DNA barcoding using standard sequencing or PCR-based techniques, permits identifying species at the larval stage (Larsen et al., 2005, Le Goff-Vitry et al., 2007a,b, Comtet et al., 2015). Thus, they offer new opportunities to examine larval abnormalities and to use early developmental stages in field studies.

During a routine analysis of meroplankton in the Gulf of Gdansk, we discovered particularly high numbers of abnormal bivalve larvae, which prompted us to assess carefully their relative abundance in a time-series sampling. In the present study, we report the occurrence of these abnormalities in larvae of four species of bivalves that were identified accurately with molecular tools. We also report abnormalities in benthic postlarvae of *Mytilus trossulus*. Based on our findings, we discuss the use of larval abnormality rates as field bio-indicators of pollution effects.

2. Materials and methods

2.1. Study area

The Puck Bay is located in the western part of the Gulf of Gdansk and is separated from the open sea by the Hel Peninsula (Fig. 1). The bay is divided by a shallow sand bar into the inner Puck Bay (known as the Puck Lagoon) and the outer Puck Bay. It is under the influence of marine waters from the deep regions of the Gulf of Gdansk and terrestrial waters, including from the Vistula, which is the longest and largest river in Poland. Water temperature in the outer Puck Bay varies between 1 and 21 °C, while the salinity reaches an average value of 7.65 (see Kruk-Dowgiałło and Szaniawska, 2008 for references). Their geographical locations and specific hydrological conditions mean the Gulf of Gdansk and the Puck Bay face significant ecological threats. Low salinity, limited water exchange, highly developed agriculture and processing, urbanized coastal zone, and river and sewage treatment plant water inflow mean that the organisms living in this area are particularly vulnerable to increasing loads of nutrients and toxic substances such as metals, organochlorine compounds, polycyclic aromatic hydrocarbons, endocrine disrupting compounds, and antibiotics (HELCOM, 2010).

2.2. Sampling

Bivalve larvae were collected from the r/v Oceanograf 2 in 2012–2013 using a WP2 plankton net with a mesh size of 100 µm at the Mechelinki site (54°36′34.40″N, 18°31′40.60″E), which is located near the mouth of the sewage outlet of the treatment plant in the village of Debogorze (Fig. 1). On each sampling date, one plankton sample was collected vertically from the bottom (depth 7–8 m) to the surface. After collection, the plankton was preserved with 96% ethanol and stored in a refrigerator until sorting. High density samples were sorted in a Motoda plankton box splitter. When available, more than one hundred bivalve larvae were then sorted randomly for further identification and examination. If the sample contained fewer individuals, all of them were analyzed. Sampling dates and the number of larvae analyzed are presented in Table 1.

A sediment sample was collected at the same site on 23/08/2012 with a Van Veen grab from onboard the r/v Oceanograf 2. Bivalves (mostly *Mytilus trossulus*) were sorted roughly and immediately preserved and stored in 96% ethanol. Later, the sample was examined to search for bivalve postlarvae. More than 100 postlarvae were sorted and easily identified as *M. trossulus* based on their morphology (e.g., Fuller and Lutz, 1989; Bownes et al., 2008; Lasota et al., 2013).

2.3. Identification of the larvae

Four bivalve species dominate in the Puck Bay: *Mytilus trossulus*, *Limecola balthica*, *Mya arenaria*, and *Cerastoderma glaucum* (e.g., Węsławski et al., 2013). Recent observations reported the occurrence of the non-native Conrad's false mussel *Mytilopsis leucophaeata* close to the sampling area (Dziubińska, 2011; Brzana and Janas, 2016) and in the Vistula Delta located in the eastern part of the Gulf of Gdansk (Brzana et al., 2017), and the non-native clam *Rangia cuneata* in the Vistula Delta and Vistula Lagoon further east (Janas et al., 2014; Warzocha

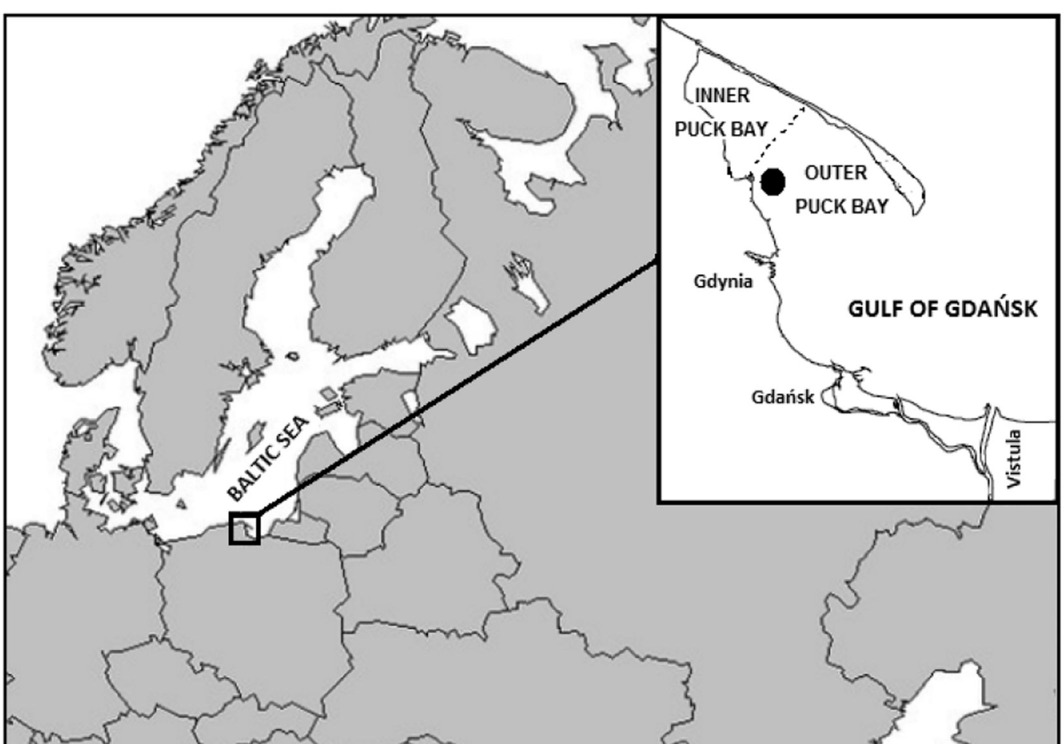

Fig. 1. Location of the sampling site (black dot).