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# Does the survivorship of activated resting stages in toxic environments provide cues for ballast water treatment?

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ABSTRACT

The toxic effects of three inorganic metals (Cu, Cr, Hg), three organic (phenol, formalin, ammonium) chemicals, ozone-enriched water and peroxides ($H_2O_2$) on embryonic development were tested in 8 species from the Porifera, Bryozoa and Crustacea. Toxicants with lower molecular weight showed stronger negative impacts on post-diapause embryos than chemicals with higher molecular weight if related to the toxicity of the chemicals to active adult stages. Only few embryos of the cladoceran *Moina macrocopa* and none of the cladoceran *Wlassicsia pannonica* treated with peroxides at concentration 0.3% developed further. Ozone-enriched water had no significant effect on post-diapause embryonic development in cladocerans. Ammonium (the product of $NH_4OH$ dissociation) in concentration 100 mg/l and higher killed all embryos of *M. macrocopa* inside protective membranes. Peroxides and ammonium are suggested for the purification of ship ballast waters as effective, non-expensive and non-persistent toxic chemicals. Resting stages of invertebrates including at least Crustaceans, Porifera and Bryozoa seem to allow not only dispersal among toxic industrial environments such as ship ballast compartments, but may also endure serious pollution events common in seaports and estuaries. *Artemia* cysts due to their strong protection against different toxic substances are recommended as a model for studies of toxic effects in diapausing stages in polluted estuaries and marine environments.

## 1. Introduction

Diapause, especially for zooplankton that produces resting eggs, is regarded as a key determinant for the dispersal ability in aquatic invertebrates (Alekseev, 1986; Cáceres and Soluk, 2002). Most invertebrates in continental waters which produce some kind of resting stages can be dispersed by natural (wind, birds ocean currents etc.) and human-mediated (ship, plane, pets and domestic animals) vectors. Natural mechanisms and vectors of dispersal of aquatic invertebrates have been discussed in detail in recent reviews by Minchin and Gollasch (2002), Bohonak and Jenkins (2003), and Havel and Shurin (2004). As an example, genetic analysis of some Caspian and North American lineages of freshwater cladocerans meanwhile indicate that human-mediated rates of species invasions are nearly 50,000 times higher than historical levels (Hebert and Cristescu, 2002).

Ship ballast water is a predominant means of global long-distance transfer of aquatic invertebrates which readily breaches geographic barriers that otherwise prevent dispersal and gene flow (Carlton and Geller, 1993). In recent decades, the role of human-mediated dispersal and transcontinental introductions has been increased, particularly due to oil transportation by large intercontinental tankers (Buchan and Padilla, 1999; Havel and Stelzleni-Schwent, 2000; Panov et al., 2004). In particular, a high biological diversity of aquatic communities has been found within ballast waters, often dominated by crustacean taxa (Carlton and Geller, 1993; Gollasch et al., 2000, 2002; Bailey et al., 2003).

Desiccation-resistant resting stages such as eggs, cysts, gemmules and statoblasts are frequently considered as an adaptation for dispersal (Karlson, 1992; Hairston, 1998; Bilton et al., 2001; Cáceres and Soluk, 2002; Panov et al., 2004; Vandekerkhove et al., 2005). Diapausing stages of aquatic organisms also facilitate the survival of resting stages under extreme conditions due to suppressed metabolism. In an anabiotic state resting stage thus can easily endure toxic conditions in a ballast water environment as well as in human modified environments of seaports commonly polluted with oil products and heavy metals. Despite this, little is known about the resistance to toxic agents in zooplankton post-diapause embryonic development when embryos are still inside protective membranes in a quiescence-like state, waiting until suitable conditions will allow a continuation of embryonic development. It has been hypothesized that survivorship of activated

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embryos could be a function of protective envelop walls mainly. However, experimental data on the viability of post-diapause embryos in aquatic invertebrates are poor (Gutierres et al., 1990).

The present study aims to fill the gap in our knowledge on tolerance in activated but cyst protected embryos to toxic substances. Data on the maximal tolerance of these embryos are required for the evaluation of a risk of alien species transportation with ship ballast water, especially collected in seaports, estuaries and other industrially polluted marine environments.

## 2. Materials and methods

We used resting stages of different aquatic invertebrates belonging to three classes: Porifera, Bryozoa and Crustacea (Table 1). Gemmulae of the sponge *Ephydatia* sp. (Demospongia), statoblasts of the bryozoan *Plumatella fungiosa* (Bryozoa) and ephippia of the crustacean *Daphnia galeata* (Daphniformes) were collected in September 2004 at a sandy beach of the Volga reservoir near Rybinsk, North-West Russia. Cysts of the fire-shrimp *Artemia salina* originated from a Crimean lagoon, Ukraine. Resting eggs of the cladocerans *Wlassicsia pannonica* (Macrothricidae), *Moina macrocopa* (Moinidae), a conchostracan *Lymnadia lenticularis* (originated from the Volga delta area) and an ostracod *Heterocypris incongruens* (collected in a temporary pool near Rybinsk, North-Western Russia) were obtained in 2004–2005 from laboratory cultures pertained over long periods of time. Most of the species used in our experiments are known from Baltic estuaries or sea lagoons (Telesh and Heerkloss, 2004).

Before the onset of experiments, diapause in resting stages had been terminated via exposing them to darkness at low temperatures (about +4 °C) within 6 months and then spent short (3–10 days) or long (27–87 days) time in +4 °C in freshwater solutions of toxic agents at different concentrations. For *Artemia salina* cysts the toxic solutions were made on the basis of artificial sea water with an intermediate salinity of 17.5 psu.

Experiments were conducted in two steps. At the first stage three inorganic (toxic heavy metal Cu, Cr, Hg) and two organic (phenol and formalin) agents at increasing concentrations of the toxicants were tested for their potential to interrupt the embryonic development of the resting stages. In all treatments each of the following concentrations was twice that of the previous one until the maximal one when the last successful embryonic developments were observed. About 1000 resting eggs of each species were treated every time. These experiments were organized by different ways of the protocol regularly used in toxicology. In the treatments with increasing concentrations of toxicants we did not calculated the mortality rate but checked if even a single resting embryo will be able to hatch after exposition to toxicants during a short (5 days) or longer term period (1–3 months). This protocol significantly increases the number of tests required for the estimation of lethal concentrations in comparison with the classical 50% lethal testing but allowed us to find the lethal concentrations by direct observation to avoid a risk of surviving in toxicant even a single embryo in large experimental groups. This provided us with more reliable information on the maximal concentration of toxicants that can overcome the resistance of protective shells and membranes in resting stages of invasive species.

Relative resistances of resting stages to toxicants in the experiments were calculated thereafter as a ratio to the maximal admitted concentrations known for active (adult) invertebrates. As reference data for resistance in adult organisms we used the Russian government's regulations on maximal admitted concentration

**Table 1**
The maximal concentration of toxic agents in which embryonic development of aquatic invertebrates were observed.

| Species | Toxic agents | Maximal admitted concentration for resting stages (MACd) (mg/l) | Non-toxic concentration of the chemical for active invertebrates NTCa (mg/l) (after Lesnikov, 1973) | Duration of stay in toxic solution (days) |
|---|---|---|---|---|
| *Ephydatia sp.* | Formalin | 155 | 0.1 | 5 |
| *Plumatella fungosa* | | 10 | | 5 |
| *Artemia salina* | | 80 | | 5 |
| *Daphnia cucullata* | | 80 | | 5 |
| *Moina macrocopa* | | 80 | | 48 |
| *Heterocypris incongruens* | | 32 | | 5 |
| *Ephydatia sp.* | $K_2Cr_2O_7$ | 125 | 0.02 | 37 |
| *Plumatella fungosa* | | 31 | | 5 |
| *Artemia salina* | | 125 | | 28 |
| *Limnadina lenticularis* | | 1000 | | 5 |
| *Daphnia cucullata* | | 1250 | | 5 |
| *Moina macrocopa* | | 500 | | 97 |
| *Moina macrocopa* | | 500 | | 5 |
| *Ephydatia sp.* | Phenol | 25 | 0.001 | 5 |
| *Plumatella fungosa* | | 62 | | 5 |
| *Artemia salina* | | 156 | | 5 |
| *Daphnia cucullata* | | 31 | | 5 |
| *Moina macrocopa* | | 31 | | 5 |
| *Heterocypris incongruens* | | 125 | | 5 |
| *Ephydatia sp.* | $CuSO_4$ | 1000 | 0.001 | 37 |
| *Plumatella fungosa* | | 125 | | 5 |
| *Artemia salina* | | 2000 | | 5 |
| *Limnadina lenticularis* | | 1000 | | 5 |
| *Daphnia cucullata* | | 1000 | | 5 |
| *Moina macrocopa* | | 500 | | 5 |
| *Moina macrocopa* | | 500 | | 97 |
| *Wlassicsia pannonica* | | 400 | | 5 |
| *Ephydatia sp.* | $HgCl_2$ | 32 | 0.00001 | 32 |
| *Moina macrocopa* | | 1 | | 32 |
| *Heterocypris incongruens* | | 15 | | 5 |
| *Plumatella fungosa* | | 32 | | 5 |
| *Artemia salina* | | 100 | | 5 |