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Mortality and histopathological effects in harbour-transplanted snails with different exposure histories

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ABSTRACT

Contaminants are important stressors in the aquatic environment and may exert selective pressures on organisms. We hypothesized that snails originating from a metal-contaminated habitat (B) would have increased tolerance to harbour contaminants (e.g. metals from antifouling paints), compared to snails originating from a relatively clean habitat (A). We assessed tolerance to metals in terms of survival and histopathological alterations after 2, 4 and 8 weeks of *in situ* exposure in three Baltic Sea boat harbours and three reference sites. We also hypothesized that any potential tolerance to contaminants would be associated with differences in genetic diversity between the two snail populations (evaluated as mitochondrial cytochrome c oxidase subunit I, COI). The results show that snails from population A survived to a higher extent compared to population B, possibly indicating either a lack of adaptation to metals in snails B or impaired health condition due to contaminant pre-exposure or a higher resilience of snails A. Moreover, the genetic diversity of COI was low within each population and did not differ between populations. In general, 83% of all the types of histopathological alterations (e.g. lysis and necrosis of gonads and digestive gland or granulocytoma and phagocytosis in the storage tissue, among others) had a higher probability of occurrence among harbour-exposed snails compared to reference-exposed snails, regardless of snail population origin. The only significant difference in histological effects between the two populations was in the frequency of parasite infestations and shell fouling, both being larger for population A than B. Interestingly, the rate of parasite infestations was higher for males than females from population A, whereas no sexual dichotomy was observed for population B. Our results show that exposure to harbour contaminants causes both lethal and sublethal toxicity to snails, and the association between many of the toxic responses and metals substantiates that antifouling substances contribute to the observed effects, although there is a large proportion of variation in our data that remains unexplained.

1. Introduction

Metal toxicity for aquatic organisms has been well-described, particularly in the case of copper-induced inhibition of algal photosynthesis or decreased fitness of invertebrates and fish (Lewis and Cave 1982). Moreover, metal contamination may represent a selective pressure for many species in different habitats, potentially leading to the elimination of individuals which cannot tolerate the metal stress. In addition, metal exposure can result in many sub-lethal effects in surviving individuals (Pease et al., 2010). Species adaptations and changes in the genetic diversity can occur in natural populations due to contaminants exposure (Bickham 2011). Specific genes can be selected for, i.e. those involved in reducing uptake or increasing the metabolism and excretion of metals. Thus, understanding the extent to which organisms adapt to contaminants is highly relevant for assessing impacts at community level (Pease et al., 2010). Contaminant exposure history is accordingly an important factor for adaptation or tolerance (Gall et al., 2013; Côte et al., 2015), although it is rarely investigated in field studies.

Field experiments are valuable tools for maintaining realistic concentrations of multiple contaminants while accounting for natural variability in environmental conditions. Caging studies have the added advantage of allowing the control of the exposure duration and proximity to site-point discharges, as well as ensuring comparable biological

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samples (Lekube et al., 2014). Histopathological assays are useful tools for detecting early signs of toxicity, as many effects of exposure will be reflected in cells and tissues earlier than mortality will occur. In molluscs, the digestive gland is the main organ involved in metabolizing contaminants and thus, alterations in digestive gland tissues are commonly used as indicators of environmental pollution (Zaldibar et al., 2008). Gastropods represent the largest group of molluscs and constitute an important part of the aquatic food chain. Although gastropods represent the most species-rich group of marine animals (Appeltans et al., 2012), they have not been monitored in the environment to the same extent as other groups of organisms. One exception to this is the investigation of TBT-induced imposex, which is widely documented in neogastropods and some prosobranch snails (Barroso et al., 2000; Strand and Jacobsen 2002).

In this study, we conducted a field exposure experiment involving translocation and caging of two populations of *Theodoxus fluviatilis* with differing exposure histories, to harbour environments which are generally affected by antifouling agents from boating activities (Warnken et al., 2004; Schiff et al., 2007). This study aims at answering two main questions: 1) which toxic effects are detectable at the tissue and cellular level in snails exposed in contaminated harbours and what are the key biotic and abiotic factors associated with these effects? and 2) does pre-exposure to contaminants affect the snails' ability to cope in polluted harbours? Specifically, we hypothesized that the incidence of both mortality and histopathological alterations would be higher amongst snails originating from a 'clean', unexposed population, compared with the pre-exposed snails which are likely to be adapted to some extent. Moreover, we hypothesized that the potential differences in responses between the two snail populations would be due to differences in genetic diversity.

2. Materials and methods

2.1. Model organism

The nerite gastropod *Theodoxus fluviatilis* is a highly abundant and widely distributed snail in the Baltic Sea (HELCOM, 2012) up to salinities of 18 psu (Skoog 1978) and it is also found in freshwaters throughout Europe (Bunje and Lindberg 2007). *T. fluviatilis* has a lifespan of 2–3 years and can reach shell lengths of up to 9 mm (Skoog 1978). The sexes are separate and the reproductive period typically peaks between May and November (Kirkegaard 2006). Eggs are laid in capsules from which miniature adults hatch (Orton and Sibly 1990). Only adult snails were used in this study and were collected at two different locations with different degrees of chemical pollution, measured here in terms of dissolved metal concentrations (Table 1). For simplicity, we name the snails originating from the relatively clean location (*Askö*), snails A and those from the more contaminated location (*Brunnsviken*), snails B. Thus, we consider snails A to have no exposure history, while snails B were pre-exposed to contaminants in their natural habitat. Snails B originated from a region with lower salinity (2.3 psu) compared to the environments in which they were subsequently transplanted and therefore, they were acclimatised over a period of three weeks in the lab (i.e. to 5.5 psu) in natural sea water.

2.2. In situ exposure

Leisure boat harbours (marinas) were chosen as representative sites of metal pollution. The exposure of the snails was carried out in three harbours in the Baltic Sea, located ca 80 km from Stockholm, Sweden (Fig. 1). For comparison, three references were also chosen; these were remote sites without harbours or other major sources of direct pollution. The snails were placed in plastic cages (400 ml) together with a piece of bladderwrack (*Fucus vesiculosus*, their natural substrate). For each snail population, five cages containing 20 snails each were placed at every location at 1 m depth. During the exposure period, the snails were feeding on the biofilm growing inside the cages. The experiment lasted 2 months (August-October), during which snail mortality was checked after 2 weeks, 1 month and 2 months, simultaneously with sampling a subset of individuals for histopathological evaluations (i.e., 2 snails per cage were sampled on the first two events and 1 snail per cage on the last sampling event). At each field visit the cages were cleaned from excessive fouling in order to ensure effective water exchange. The experimental design is illustrated in Fig. 2. Water samples were collected from a depth of 1 m with a Ruttner sampler and sediment was collected with an Ekman grab sampler. All the samples were placed in acid-washed plastic containers and transported to the laboratory in cooling boxes and stored at 4 °C until analysis.

2.3. Analysis of metals and nutrients

Water samples from each location were analysed for nutrients: NO_2 + NO_3 according to ISO 13395 and PO_4 according to ISO 15681-2, using an Autoanalyzer II, Technicon. Dissolved metals (Cu, Zn, Pb) were analysed in filtered (0.45 µm) samples (1–3 samples per site) by Inductively-Coupled Plasma Mass Spectrometry, ICP-MS in a Thermo Scientific X-series 2 according to SS EN ISO 17294-2:2005. Metal concentrations (Cu, Zn and Pb) in the top 2 cm of sediment, approximately, were analysed by ICP-SFMS (Sector-Field Mass Spectrometry) according to SS EN ISO 17294-1,2 mod.; QA/QC information is presented in supplementary Table S1. Cu and Zn were chosen due to their high prevalence in antifouling paints, whereas Pb was analysed in order to get an indication of anthropogenic pollution unrelated to boating activities. In a previous study (under review), we also measured organic AF biocides, namely mono-, di- and tributyl tin, irgarol and diuron in the sediments, and as the levels were generally low or below the limit of

Table 1
Metal concentrations in the water and sediment collected from reference sites and harbours; N/A is not measured, TS = total solids, LOQ = limit of quantification; N for dissolved metals was 3 for Marina 1 and Guest harbour, 2 for Marina 2 and 1 for the others.

	Metals in water (µg/L)			Metals in sediment (mg/kg TS)			
Site	Cu	Zn	Pb	Cu	Zn	Pb	TS
prior exposure							
Askö	0.92	5.31	0.08	N/A	N/A	N/A	N/A
Brunnsviken	1.54	22.3	0.61	N/A	N/A	N/A	N/A
in situ exposure							
Marina 1	3.49	10.55	0.05	61.2	162	19.7	36.7
Marina 2	2.65	8.05	0.06	23	69.2	11.3	51.2
Guest harbour	1.77	3.27	0.15	33.4	101	23.2	56.2
Reference 1	0.752	1.86	< LOQ	3.81	162	1.57	74.7
Reference 2	0.953	0.846	< LOQ	15.2	74.6	6.52	45.6
Reference 3	0.62	3.35	< LOQ	5.5	16.6	2.44	88.4

Standard deviations for dissolved Cu, Zn and Pb, respectively are 0.28, 2.18, 0.02 µg/L (Marina 1) and 0.25, 0.91, 0.09 µg/L (Guest harbour).