A multilevel approach to predict toxicity in copepod populations: Assessment of growth, genetics, and population structure

Johanna Gardeström [a,*], Elena Gorokhova [a], Michael Gilek [b], Mats Grahn [b],
Bengt-Erik Bengtsson [c], Magnus Breitholtz [c]

[a] *Department of Systems Ecology, Stockholm University, SE-10691 Stockholm, Sweden*
[b] *School of Life Sciences, Södertörn University College, SE-14189 Huddinge, Sweden*
[c] *Department of Applied Environmental Science (ITM), Stockholm University, SE-10691 Stockholm, Sweden*

Abstract

One of the goals of environmental risk assessment (ERA) is to understand effects of toxicant exposure on individual organisms and populations. We hypothesized that toxicant exposure can reduce genetic diversity and alter genotype composition, which may ultimately lead to a reduction in the average fitness of the exposed population. To test this hypothesis, we exposed a copepod, *Nitocra psammophila*, to a toxic reference compound and assayed resulting alterations in genetic structure, i.e. expected heterozygosity and percent polymorphic loci, as well as other population- and fitness-related measures, i.e. population abundance, demographic structure and juvenile growth. The copepods were exposed to $0.11–1.1\,\mu g$ of the pentabromo-substituted diphenyl ether (BDE-47) mg^{-1} freeze-dried algae for 24 days (i.e. >1 generation). There was no significant decline in total population abundance. However, there were significant alterations in population structure, manifested as diminished proportion of nauplii and increased proportion of copepodites. In addition, individual RNA content in copepodites decreased significantly in exposed individuals, indicating declined growth. Finally, in the exposed populations, heterozygosity was lower and genotype composition was altered compared to the controls. These results therefore confirm the hypothesized reduction in overall genetic variability resulting from toxicant exposure. Multilevel approaches, such as the one used in the present study, may help unravel subtle effects on the population level, thus increasing the predictive capacity of future ERA.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Environmental risk assessment; Multilevel; Toxic exposure; Crustacea; Population genetics; RNA

1. Introduction

Predicting impacts of anthropogenic chemical substances on ecosystems is one of the main goals of environmental risk assessment (ERA). Usually, ERA is based on a bottom–up methodology, where effects at higher organization levels are extrapolated from results of standardized toxicity tests (European Commission, 2003). These tests often aim to identify effects on individual organisms, most often short-term lethal effects (i.e. LC/EC_{50}) but sometimes also chronic no-observed-effect concentrations (NOECs; Forbes et al., 2001). However, extrapolating from the individual to the ecosystem level is a complex task, because the connections between these levels are far

from straightforward and sometimes even misleading (Calow et al., 1997; Belfiore and Anderson, 2001; Forbes and Calow, 2002). Furthermore, it has been suggested that population level responses need to be included in ecotoxicological tests to allow better predictions of effects and risks at the ecosystem level (Bechmann, 1994; Calow, 1996; Calow et al., 1997; Forbes et al., 2001; Forbes and Calow, 2002). This means that developing testing strategies that include scientifically based combinations of species and endpoints is essential for evaluating the complexity of potential biological and environmental effects at a reasonable financial cost (e.g. Escher and Hermens, 2002).

Biochemical markers together with biological indicators at higher levels of biological organization can provide good measures of altered state in individual organisms and populations evoked by exposure to toxicants. At the perspective of the individual organism level, the effects of a toxicant can be tackled by behavioral mechanisms (e.g. avoidance) or by cellular mecha-

---

* Corresponding author. Tel.: +46 8 16 37 04; fax: +46 8 15 84 17.
E-mail address: johanna@ecology.su.se (J. Gardeström).

nisms, such as biochemical and physiological responses (Van Straalen, 2003). The latter allows for acclimatization at the cost of important fitness traits such as growth and fecundity (Futuyma, 1997). Measurements of metabolic enzyme activities and RNA-based indices have been especially valuable as indicators of condition in studies of marine invertebrates and fish, i.e. groups for which accurate determination of metabolic and growth rates are difficult (Dahlhoff, 2004). At the population level, tolerance can be developed by genetic adaptation (Van Straalen, 2003), which may lead to alterations in the genetic composition and genetic diversity of the population. Pollution is a well-documented selective force that has been found to, e.g., induce metal tolerance in plants (e.g. Bradshaw and McNeilly, 1981) and pesticide resistance in insects (e.g. Devonshire et al., 1998). More recently, pollution tolerance has been detected in a variety of aquatic organisms (Tanguy et al., 1999; Belfiore and Anderson, 2001). The reduction in overall genetic variability is a frequently observed phenomenon (Ma et al., 2000; Van Straalen and Timmermans, 2002; Ross et al., 2002), even when the population abundance remains unaltered (Street and Montagna, 1996). It can result from a genotoxicant-induced selection at loci important for survival and propagation in polluted environments or from a genetic drift, e.g. as a result of a population bottleneck (Staton et al., 2001), and might ultimately lead to a reduction in the average fitness of the exposed populations (Bickham et al., 2000).

Populations of the meiobenthic harpacticoid copepod *Nitocra spinipes* have been observed to exhibit decreased mean juvenile developmental rate when exposed to brominated flame-retardants and synthetic musk fragrances (Breitholtz et al., 2003; Breitholtz and Wollenberger, 2003). In these studies, the pollutant-exposed populations sustained the ability to maintain a stable population growth. It was suggested that the observed phenomenon could be attributed to compensatory effects (i.e. decreased competition among surviving individuals and/or shortened inter-clutch period). This could lead to differential survival and reproduction of tolerant individuals, i.e., genetic adaptation at the cost of the decreased effective population size. As a result, the genetic variability within these toxicant-tolerant populations could have been reduced, through decreased heterozygosity compared to the control populations.

The aim of this study was to: (1) test the hypotheses that toxicant exposure can reduce expected heterozygosity and genetically differentiate populations and (2) compare these population genetic endpoints with other population- and growth-related measures, i.e. population abundance, demographic structure and juvenile growth. To investigate this, we exposed *Nitocra psammophila* to the polybrominated diphenyl ether (BDE-47) in a laboratory test that lasted longer than a generation time.

2. Materials and methods

2.1. Test organisms

In general, harpacticoid copepods have short generation times, and thus they can be easily studied for the duration of a full life cycle, focusing on all crucial ontogenetic stages and life history traits, such as larval development, reproduction, etc.; this is essential for characterization of both lethal and sublethal effects of toxicants (Ingersoll et al., 1999). Harpacticoid copepods have therefore been extensively used as test organisms in chronic ecotoxicity testing (e.g. Hutchinson et al., 1999; Chandler et al., 2004; Breitholtz et al., 2003; Breitholtz and Wollenberger, 2003). The reason for using *N. psammophila* in this study rather than *N. spinipes* (which is used in standardized toxicity tests) was that population of the former species has been cultured for a shorter period and therefore presumably exhibits higher levels of heterozygosity compared to the latter species that has been kept in the laboratory for more than 30 years, which implies a high level of inbreeding. It was necessary to collect the *N. psammophila* almost 1 year before the beginning of this study since it takes time to isolate and identify a single species that can handle laboratory conditions.

Ovigerous *N. psammophila* were isolated at Utansjö (62°46′N, 17°57′E) on the East coast of Sweden in August 2002. Cultures of this species have since then been maintained in laboratory at 20 °C, 3.5‰ salinity, in darkness, and fed with a mixture (1:2 on a weight basis) of freeze-dried algae (diatom *Thalassiosira weissflogi* and cryptophyte *Rhodomonas baltica*). The experiment was conducted between 17th of June to 11th of July in 2003.

2.2. Test substance

Professor Åke Bergman at the Department of Environmental Chemistry, Stockholm University, kindly provided the polybrominated diphenyl ether 2,2′,4,4′-tetrabromo-diphenyl ether (BDE-47). This flame retardant had a purity of >99% and was chosen as a model substance since it is a common environmental pollutant worldwide (e.g. De Wit, 2002). Additionally, this substance was suggested to cause compensatory effects in populations of *N. spinipes* (Breitholtz and Wollenberger, 2003).

2.3. Experimental design

For the purpose of the present study, the BDE-47 dose range was intended to comprise a high dose (x) that should give significant effects on population dynamics, and two lower doses (i.e. $x/10$ and $x/100$) that should not give any apparent effects on the population dynamics. Another prerequisite for our testing was the recommendation by Breitholtz and Wollenberger (2003), who tested the same diphenyl ether for chronic effects, to not normalize effect levels of very hydrophobic substances to their concentration in the water since they partition almost exclusively to non-water phases; only 0.5% was present in filter-(0.3 μm)-passing test medium after 15 days exposure although 70% of the test medium was renewed every second day. We therefore decided to administer the diphenyl ether as a single dose normalized to the amount of freeze-dried algae. Further, renewal of test medium containing substances that partition to non-water phases will result in increased load of the substance in the test system over time (Breitholtz and Wollenberger, 2003). Hence,