Effects of the polycyclic musk HHCB on individual- and population-level endpoints in *Potamopyrgus antipodarum*

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Abstract

Although the polycyclic musk 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran (HHCB) is frequently detected in aquatic sediments, very little is known about its effects on sediment-feeding organisms. Effects of sediment-associated HHCB on growth, feeding rate, survival and reproduction in the gastropod *Potamopyrgus antipodarum* were measured in the laboratory. Snails were exposed to six nominal HHCB concentrations: 0, 0.1, 1, 10, 30 and 100 μg g^{-1} dry weight (dw) sediment. Adult survival and growth were not affected by HHCB. However, juvenile growth and survival, reproduction, time to first reproduction and adult feeding rate were more sensitive endpoints and declined with increasing HHCB concentration. Individual-level endpoints for *P. antipodarum* were integrated into a population model to investigate the effects of HHCB on population growth rate. Under otherwise favorable laboratory conditions, population growth rate was slightly (by ca. 2%), but not significantly, reduced with increasing HHCB exposure concentration. Model simulations were performed to explore the consequences of HHCB exposure under more ecologically realistic conditions (i.e., survival and reproduction of unexposed snails were markedly reduced relative to the laboratory). The results suggest that despite detectable effects of HHCB on individual-level endpoints measured in the laboratory, impacts on population dynamics of this deposit feeder are not likely to occur at environmentally relevant exposure concentrations.

Keywords: Deposit feeder, Life-table response experiment, Elasticity, Fragrance materials, Population growth rate, Sediment

1. Introduction

Although toxicant effects are most frequently measured in terms of individual performance, it is the impacts on populations and the communities and ecosystems of which they are a part that are of primary concern for risk assessment (Forbes *et al.*, 2006). One of the main difficulties in assessing the ecological risks of toxic substances is in linking effects on individual-level endpoints measured in toxicity tests to ecologically relevant impacts (Levin *et al.*, 1996; Forbes and Calow, 2002). It has been shown that there is not necessarily a simple link between effects measured at the individual level and effects occurring at the population level (Kammenga *et al.*, 1996; Forbes and Calow, 1999). A large number of studies have demonstrated the value of using demographic models to link individual- to population-level effects of chemicals and other stressors (Hansen *et al.*, 1999a, 1999b; Linke-Gamenick *et al.*, 1999; Caswell, 2001; Widarto *et al.*, 2004; Barnthouse *et al.*, 2008; Akçakaya *et al.*, 2008).

The present study was designed to quantify effects of the polycyclic musk fragrance 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran (HHCB) on the deposit-feeding gastropod, *Potamopyrgus antipodarum*. More specifically, chronic effects of sediment-bound HHCB were measured on different individual life-history traits (juvenile and adult survival, time to first reproduction, size at first reproduction and reproductive output), as well as on individual growth and feeding rate. Subsequently, the measured effects on the individual life-history traits were integrated into a population model to assess the likely risks of HHCB for *P. antipodarum* population dynamics.

HHCB is a polycyclic musk fragrance that is used in various products, including cosmetics, detergents, shampoos, soap, washing powder and household-cleaning products (Balk and Ford, 1999a, 1999b; Balk *et al.*, 2004; Reiner and Kannan, 2006). HHCB is one of the most widely used polycyclic musks (Rimkus, 1999), representing (together with 6-acetyl-1,1,2,4,4,7-hexamethyltetraline (AHTN)) about 95% of the total market volume of this group of chemicals (Balk and Ford, 1999b; Rimkus, 1999). Though the use of HHCB in Europe appears to be slightly declining, it is still substantial and was 2400 tons in 1992 (Plassche and Balk, 1997), 1481 tons in 1995, 1473 tons in 1998 (Balk *et al.*, 2004) and 1427 tons in 2000 (HERA, 2004). After use most of the HHCB is released down

the drain to wastewater treatment plants. Consequently, HHCB has been detected in sewage sludge, influent and effluent (Simonich *et al.*, 2002; Berset *et al.*, 2004; DiFrancesco *et al.*, 2004; Kupper *et al.*, 2004; Bester, 2005; Horii *et al.*, 2007; Reiner *et al.*, 2007). The release to wastewater treatment plants is HHCB's primary route into the environment, since it is not completely removed during the treatment process. The continuous production and use of HHCB result in a constant input into wastewater treatment plants and thereby a steady input into the aquatic environment.

The first reported occurrence of polycyclic musks, including HHCB, in different environmental compartments was published by Esche *et al.* (1994). Subsequently, HHCB has been reported in lake and river water (Peck and Hornbuckle, 2004; Moldovan, 2006), in the North Sea (Bester *et al.*, 1998), in biota such as mussels and fish (Fromme *et al.*, 2001a; Gatermann *et al.*, 2002) as well as in top predators, e.g. porpoises, sharks, otters, seals and dolphins (Kannan *et al.*, 2005; Nakata *et al.*, 2007).

HHCB has been found in suspended particulate matter and in sediment at a concentration range 0.002–2.2 μg HHCB g^{-1} dry weight (dw) sediment (Lach and Steffen, 1997; Fromme *et al.*, 2001b; Dsikowitzky *et al.*, 2002).

In the present study we exposed a deposit-feeding gastropod to sediment-associated HHCB. Given its route of entry into the environment and its hydrophobic properties (log K_{ow} = 5.9), sediment is likely to be an important environmental compartment for HHCB. Since deposit feeders use sediment as their food source and ingest one to many times their own body weight in sediment daily (Lopez and Levinton, 1987), these animals are particularly susceptible to exposure to sediment-bound toxicants. Because deposit feeders represent an important link in aquatic food chains and play a key role in the recycling of organic matter, impacts on the populations of such organisms are of concern (EC, 2003).

2. Methods

2.1. Study organism

P. antipodarum is a deposit-feeding gastropod that inhabits the upper layers of freshwater and brackish sediments. *P. antipodarum* was introduced from New Zealand to Europe in the late 1800s and has since spread throughout most of Europe (Ponder, 1988; Jacobsen *et al.*, 1996). More recently the species has been found in the western United States (Hall *et al.*, 2006).

In Europe *P. antipodarum* reproduces by parthenogenesis, and populations generally consist of a single genetic clone (Jacobsen *et al.*, 1996). Snails start to reproduce when they reach a shell length of 3.1–4.0 mm (Dahl and Winter, 1993; Jensen *et al.*, 2001), and adults may reach a shell length of up to 6 mm. *P. antipodarum* is ovoviviparous, and the duration of the embryonic stage is 30–35 days at 15 °C (Dorgelo, 1991). *P. antipodarum* and several closely related species of the genus *Hydrobia* are important representatives of temperate surface-deposit-feeding species.

P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 °C on natural pre-frozen sediment (< 250 μm) and artificial freshwater (192 mg/L NaHCO$_3$, 8 mg/L KCl, 245 mg/L MgSO$_4$ and 120 mg/L CaSO$_4$ in de-ionized water, pH 7.2 (Jensen *et al.*, 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.

2.2. Preparation of sediment

All sediments used in the experiments were collected from a shallow area in Roskilde Fjord away from potential point sources of pollutants (Store Havelse, Roskilde fjord, Denmark). The top 5 cm of the surface sediment were collected and sieved (< 63 μm) using de-ionized water and then frozen until use (–20 °C). The sieved sediment had a water content of 71.6 ± 0.15% (24 h at 105 °C; n = 3), an organic content of 11.7 ± 0.13% (6 h at 550 °C; n = 3) and a carbon content of 5% ± 0.2% (EA 1110 CHNS-elemental analyzer; n = 3). The nominal concentrations of HHCB were 0 (= control), 0.1, 1, 10, 30 and 100 μg HHCB g^{-1} dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of *P. antipodarum* determined in a 2-week pilot experiment.

Unless otherwise indicated, concentrations of HHCB are presented as nominal concentrations.

An HHCB-contaminated stock sediment (500 μg HHCB g^{-1} dw sediment was made by adding a known amount of fluid HHCB to a glass container followed by a known volume of wet sediment. Subsequently, the glass container was shaken for 24 h in the dark. The five HHCB treatments were made by adding a known amount of wet stock sediment to a known volume of non-spiked wet sediment in a glass container. The glass containers were left on a shaking table for 24 h in the dark. Nominal HHCB concentrations were estimated by accounting for water content of the sediment. The sediment was homogenized before being divided into smaller portions and subsequently frozen until use (–20 °C).

2.3. Extraction and analysis of HHCB

Sediments were extracted using an accelerated solvent extraction system, ASE200 (Dionex, Hvidovre, Denmark) based on the method used by Difrancesco *et al.* (2004) and Simonich et al (2000) with some modifications. The sediment samples were homogenized using a hand-held mixer. The water content was determined by drying the sediment at 105 °C for 15 h. Approximately 2 g sediment was spiked with acetyl cedrene (as an internal standard) and thoroughly mixed with 2 g Hydromatrix (Varian, Palo Alto, CA, USA) to create a free-flowing mixture. Hydromatrix absorbs water from the sediment, creates a larger surface area, reduces clumping and facilitates even dispersal of the solvent in the sediment. For each sample, a 33 ml extraction cell was used, which was packed with 3 g silica gel (activated for 15 h at 105 °C) as a clean up step. The sediment–Hydromatrix mixture was added to the cell, and the cell was filled with Hydromatrix approximately 1 cm from the top and packed tightly. Triplicates were made for all concentrations.

The cells were extracted with 30 ml dichloromethane (DCM) at 60 °C and 2000 psi (138 bar) in static mode for 15 min. The extraction took approximately 45 min per sample.

Sodium sulfate was added to the extracts from the three highest concentrations (10, 30 and 100 μg HHCB g^{-1}) to bind water. The sodium sulfate was allowed to settle, and the extracts were transferred to GC–MS vials.

One milliliter toluene was added to the extracts from 0, 0.1 and 1 μg HHCB g^{-1} dw sed, which were evaporated to a total volume of 1 ml (SE 500, Dionex, Hvidovre, Denmark) and transferred to GC–MS vials.

All extracts were analyzed by GC–MS using an Agilent 6890 GC-5973 MS equipped with a J&W DB-1701 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 7683 autosampler. Samples were analyzed using selective ion monitoring, and each compound was identified and quantified based on a quantification ion (243) and two confirmation ions (213 and 258). The ionization mode was electron impact (70 eV), the ion source temperature was 230 °C and the dwell time was 25 ms/ion. The GC temperature program was isothermal at 35 °C for 2 min, 5 °C/min to 200 °C and 20 °C/min to 280 °C for 2 min. All samples were analyzed on the GC–MS using a split/splitless injector in splitless mode.

2.4. Experimental design

To investigate the effects of HHCB on selected life-history traits and feeding rate, snails were exposed individually with 10 replicates per treatment. The snails were exposed in 5 ml Nunclon multi-well dishes (NUNC A/S, Roskilde, Denmark) containing 0.5 ml wet sediment (≈0.3 g dw sediment) and 2.5 ml oxygenated artificial freshwater. Wells were monitored once a week, and snails were transferred to new wells containing fresh experimental sediment. The wells were prepared 1 day before they were used for the experiment and left in the dark overnight. The overlying water was renewed with clean oxygenated water 1 h prior to transferring the snails to the wells. The lids on the wells were covered with wet paper towels and aluminum foil to minimize water evaporation during the experiments. The experiments were conducted in the dark at 17 °C and 2 ml of the overlying water was renewed every second day to avoid oxygen deficiency.

Due to the long lifespan of *P. antipodarum* (16–17 months) the experiment was divided into two parallel experiments: (i) juveniles exposed from birth to first reproduction to measure juvenile survival, growth, time to first reproduction and size at first reproduction and (ii) adults exposed for 12 weeks to measure growth rate and reproduction. An additional 6-week experiment was carried out with adult snails to measure HHCB effects on feeding rate.

2.4.1. Juvenile growth, survival, time to first reproduction and size at first reproduction

Sixty adult snails were transferred to a 1-L aquarium with 0.5 cm sediment and allowed to reproduce for 1 week, where after they were removed from the aquarium. After 8 weeks, juveniles were collected with a pipette from the aquarium, randomly placed individually in dishes with clean artificial fresh-

water and videotaped to measure shell length. Subsequently, the snails were transferred individually to wells containing experimental sediment as described above.

A video camera mounted on a dissection microscope was used to record snails, and individual shell length (L) was measured using an image analysis program (SigmaScan®Pro. version 5.0, Jandel, Erkrath, Germany). Juvenile snail shell lengths were measured every 3 weeks over a 12-week period. Specific growth rates (SGRs) from week 0 to week 12 were estimated following Kaufmann (1981):

$$SGR = \frac{\ln S_2 - \ln S_1}{t_2 - t_1}$$

where S_1 and S_2 are the shell lengths at time t_1 (week 0) and t_2 (week 12), respectively.

Survival was recorded each week when the snails were transferred to new wells. After 30 weeks, which is close to the minimum age at first reproduction under these conditions (Jensen *et al.*, 2001), the wells were examined for offspring every week until almost all snails had reproduced by week 43. There were two individuals that did not reproduce (one at 30 µg HHCB g^{-1} and one a 100 µg HHCB g^{-1}), and these were not included in the analysis of time to first reproduction. At the time to first reproduction, shell lengths of the reproducing snails were measured as described above.

2.4.2. Adult growth, survival and reproduction

Adult snails of a similar size (4.078 ± 0.090 mm) were collected from cultures, shell lengths were measured and the snails were randomly transferred individually to wells containing experimental sediment. The shell lengths were measured every 3 weeks over a 12-week period, and SGRs were calculated as described above. Each week over a 10-week period, when the snails were transferred to new wells, survival was recorded and reproduction was estimated by counting the number of offspring produced per female using a dissecting microscope.

2.4.3. Feeding rate

Adult snails in the size range 4.162 ± 0.133 mm were collected from cultures and randomly transferred individually to wells with experimental sediment. Feeding rate was measured as the dw of fecal pellets produced per unit time, since nearly all the sediment deposit feeders ingest consists of indigestible mineral grains that pass though the alimentary tract without loss (Lopez and Levinton, 1987). Each week for 6 weeks the fecal pellets from individual adult snails were sieved from the sediment with an 80 µm sieve and rinsed with de-ionized water. The pellets were dried at 105 °C for 24 h and subsequently weighed.

2.5. Demographic analysis

A simple two-stage demographic model was used to determine the population growth rate (λ) and to estimate the effects of HHCB at the population level. The two-stage model distinguishes between juvenile and adult life stages and integrates five life-history traits; juvenile and adult survival, reproduction, time to first reproduction and time between reproductive events (Calow *et al.*, 1997).

F is the number of female offspring produced per female per time unit (i.e., per week; in this case averaged for weeks 5–10 of the experiment as explained below), and p_j represents the survival of juveniles between time units from birth to first reproduction. To simplify the mathematics an additional variable is defined, $S_j = p_j^{t_j}$, which represents juvenile survival from birth to age t_j, S_a represents average adult survival between time units, t_j is the time to first reproduction and t_a the time between reproductive events. In this experiment t_a was set to 1 week for all treatments since reproduction was measured once a week.

$$1 = FS_j\lambda^{-t_j} + S_a\lambda^{-t_a}$$

The demographic sensitivities and elasticities of the life-history traits were calculated according to Sibly *et al.* (2000) to estimate the effect on λ of absolute and proportional changes in life-history traits, respectively. The standard error and confidence interval (CI) for λ were estimated following Lande (1988) and Sibly *et al.* (2000). Furthermore, decomposition analyses were carried out to quantify the relative contribution of each of the affected life-history traits to the observed impact on λ (Caswell, 1996; Levin *et al.*, 1996).

2.6. Statistical analyses

Differences in starting shell length, SGR, average weekly reproduction, average weekly feeding rate, size at first reproduction and time to first reproduction as a function of HHCB concentration were tested using one-way ANOVA. In cases where significant or marginally significant effects were detected for one-way ANOVA, two-sided Dunnett's tests were performed to compare the con-

trol with each of the treatments. For feeding rate a two-way ANOVA was used to test for interaction effects between exposure time and exposure concentration. In the event of no interaction a reduced model (without the interaction term) was used to test whether time and exposure concentration had significant effects on feeding rate. Data are presented as mean ± 95% CI. Values of λ were considered to be significantly different if their 95% CIs did not overlap (Sibly *et al.*, 2000). A significance level of $p \leq 0.05$ is used throughout. *P*-values between 0.05 and 0.1 were defined as marginally significant.

3. Results

3.1. Extraction and spiking efficiencies

The extraction recoveries of HHCB for the different treatments are presented in Table 1. We observed recoveries between 64% and 86%.

3.2. Survival

Adult survival (S_a) was not affected by HHCB, and all adults survived the entire exposure period. Juvenile survival (S_j) decreased with increasing HHCB concentration. One hundred percent of the juveniles exposed to 0, 0.1, 1 and 10 µg HHCB g^{-1} survived from birth to first reproduction. Juvenile survival from birth to first reproduction was 90% at 30 µg HHCB g^{-1} and 80% at 100 µg HHCB g^{-1}.

3.3. Growth

SGRs for juvenile and adult snails are shown in Figure 1. The initial average shell lengths were 4.078 ± 0.090 mm for adults and

Table 1. Recovery concentrations and recovery (%) from the extraction of different treatments (mean ± SD)

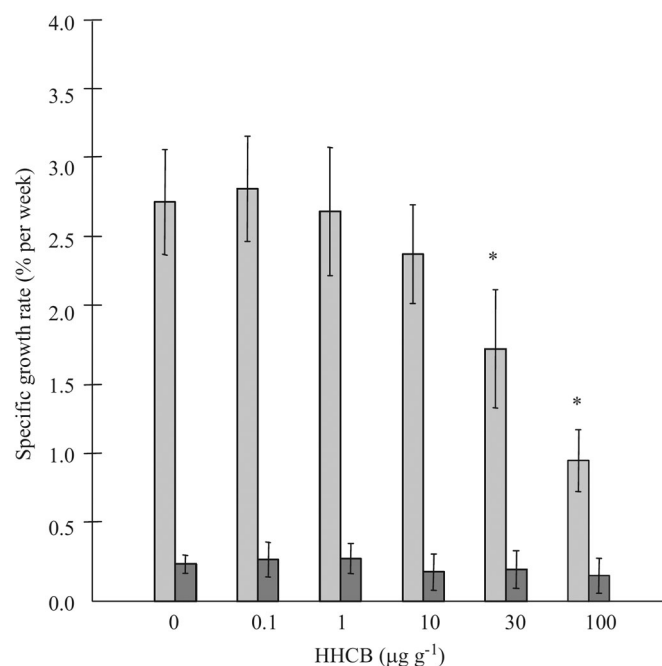| Treatments (µg g^{-1})	Recovery concentrations (µg g^{-1})	Recovery (%)
0.1	0.086 ± 0.008	86.3 ± 7.9
1	0.810 ± 0.048	81.0 ± 4.8
10	7.0 ± 0.2	71.8 ± 3.2
30	19.3 ± 0.9	64.5 ± 2.8
100	64.2 ± 2.0	64.2 ± 2.0

Figure 1. Specific growth rates (SGR) for juvenile (light bars) and adult (dark bars) snails.
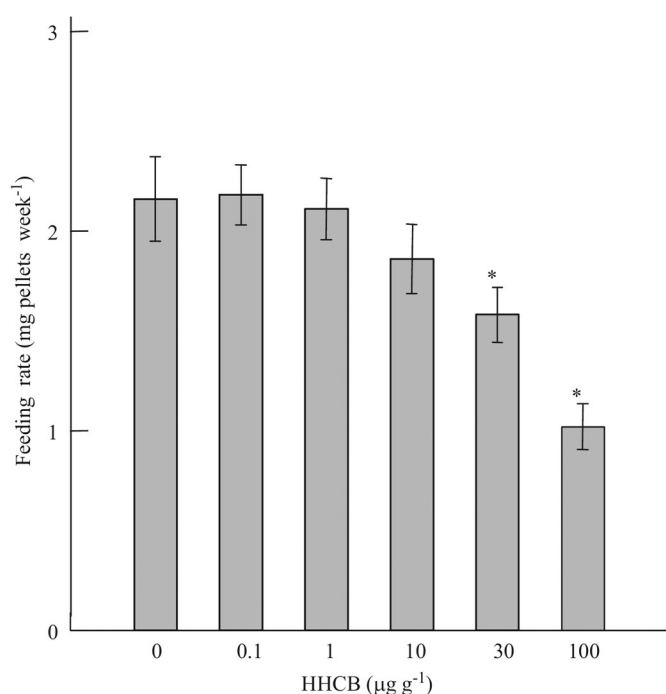
Figure 2. Average adult weekly feeding rates (averaged over all weeks for each snail) for different treatments.
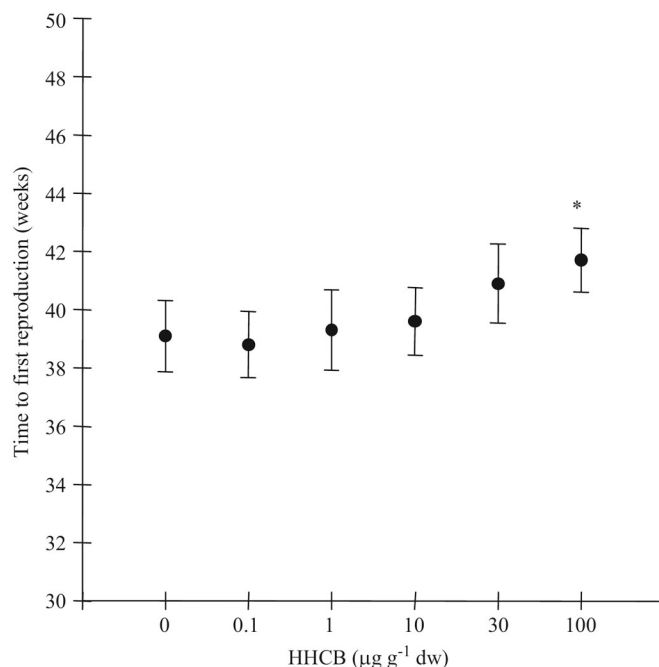

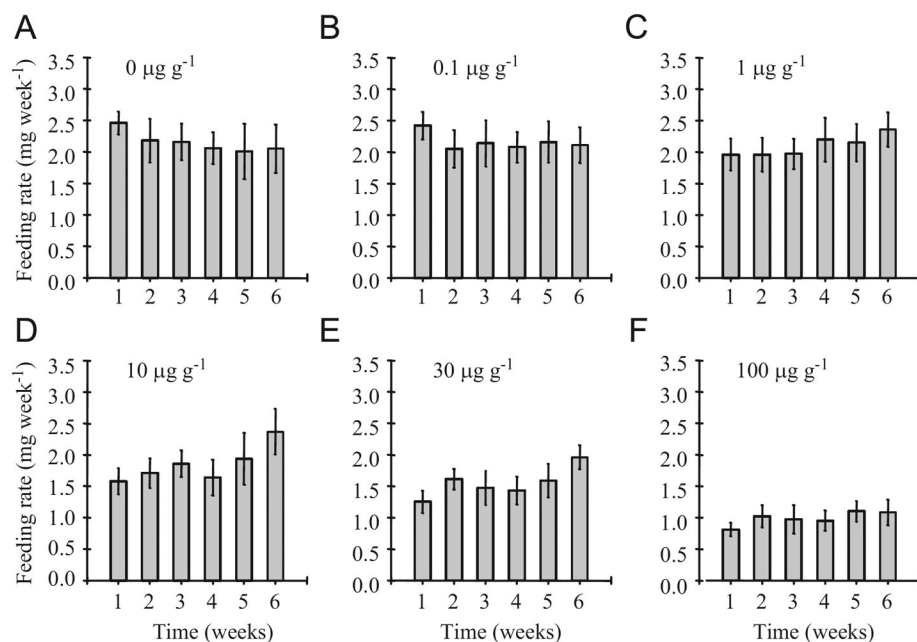**Figure 4.** Time to first reproduction (t_j) as a function of HHCB concentration.

Figure 3. Feeding rates over time in the treatments (A) 0, (B) 0.1, (C) 1, (D) 10, (E) 30 and (F) 100 µg g^{-1}.

1.059 ± 0.099 mm for juveniles. There were no significant differences in shell length among exposure groups at the start of the experiment for juvenile or adult snails ($p = 0.960$; $p = 0.985$, respectively). During 12 weeks of exposure HHCB had no significant effect on adult SGR ($p = 0.600$) but significantly reduced juvenile SGR ($p < 0.001$). Juvenile snails exposed to 30 µg HHCB g^{-1} ($p < 0.001$) and 100 µg HHCB g^{-1} ($p < 0.001$) had significantly slower growth rates compared to the control group.

3.4. Adult feeding rate

Average adult weekly feeding rates (averaged over all weeks for each snail) for the different treatments are shown in Figure 2. HHCB af-

fected average adult feeding rate ($p < 0.001$), which was significantly reduced in snails exposed to 30 µg HHCB g^{-1} ($p < 0.001$) and 100 µg HHCB g^{-1} ($p < 0.001$) compared to the control group.

Feeding rates over time in the different treatments are presented in Figures 3A–F. There was a significant interaction effect between time and exposure concentration on feeding rate, indicating that the response of *P. antipodarum* to HHCB was time dependent. A graphical comparison among treatments shows that for the control treatment and 0.1 µg HHCB g^{-1} there appeared to be no change in feeding rate over time (Figures 3A and B). For 1 µg g^{-1} there was a slight increase in feeding rate over time (Figure 3C). This increase was more apparent at 10 and 30 µg HHCB g^{-1} (Figures 3D and E). Time did not seem to affect the feeding rate in snails exposed to

100 μg HHCB g^{-1} (Figure 3F). However, the average feeding rate was much lower in these snails compared to the control treatment and snails exposed to lower concentrations of HHCB (Figure 2).

3.5. Time to first reproduction

Time to first reproduction (t_i) as a function of HHCB concentration presented in Figure 4 is significantly affected by HHCB ($p = 0.0001$), and was marginally longer in snails exposed to 30 μg HHCB g^{-1} ($p = 0.078$) and significantly longer at 100 μg HHCB g^{-1} ($p = 0.005$) compared to the control group.
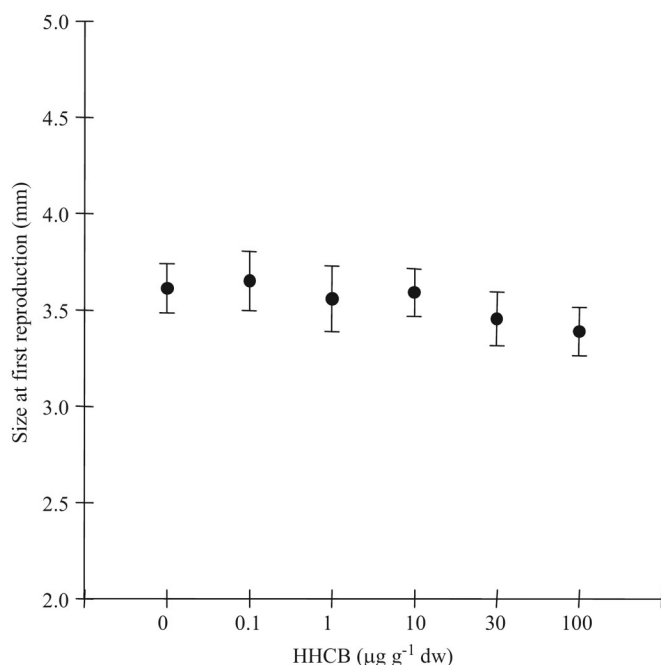

Figure 5. Size at first reproduction vs HHCB concentration.

3.6. Size at first reproduction

Overall, size at first reproduction seemed to decrease with increasing HHCB concentration (Figure 5). However, this effect was only marginally significant ($p = 0.051$) and it was only snails in the highest HHCB concentration that differed marginally from the control group ($p = 0.075$). There were no significant differences in size between the other exposure groups and the control snails (all $p > 0.1$).

3.7. Reproduction

The relationship between reproductive output and HHCB exposure over time (i.e., 10 weeks) is presented in Figures 6A–F. It appears that there is a decline in reproduction over time, but the pattern is not very clear. However, looking at the graphs for each HHCB treatment (Figures 6A–F), it seems that reproduction on average is higher during the first 4 weeks compared to the last 6 weeks especially for snails exposed to the higher HHCB concentrations. *P. antipodarum* is ovoviviparous, and the duration of the embryonic stage is 30–35 days at 15 °C (Dorgelo, 1991). Therefore the first four experimental weeks represent juveniles that developed prior to the start of the experiment. We therefore chose to analyze the data for the first 4 weeks and the remaining 6 weeks separately. Whereas there were no among-treatment differences in reproduction during the first 4 weeks of exposure ($p = 0.724$), a significant effect was detected ($p = 0.043$) on reproductive output averaged over the last 6 weeks (Figure 7). Reproduction was significantly reduced at 10 ($p = 0.028$), 30 ($p = 0.004$) and 100 ($p = 0.001$) μg HHCB g^{-1} dw sediment compared to the control group.

3.8. Population growth rate

For all treatments the population growth rate (λ) was greater than one, which indicates a population increasing in size (Figure 8); λ slightly decreased with increasing HHCB concentration,
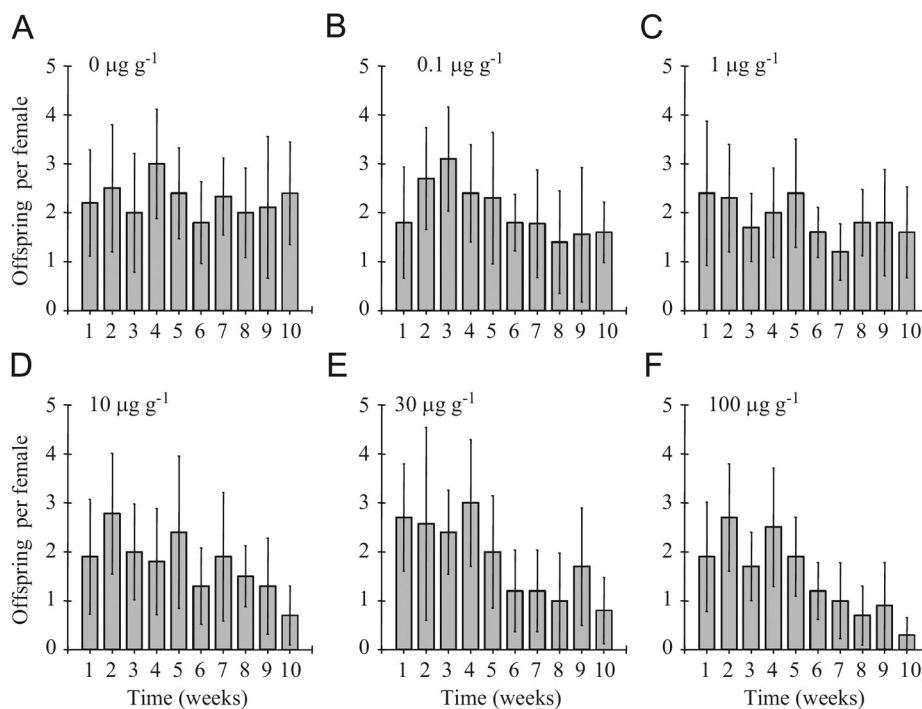

Figure 6. Relationship between reproductive output and HHCB exposure over time: (A) 0, (B) 0.1, (C) 1, (D) 10, (E) 30 and (F) 100 μg g^{-1}.
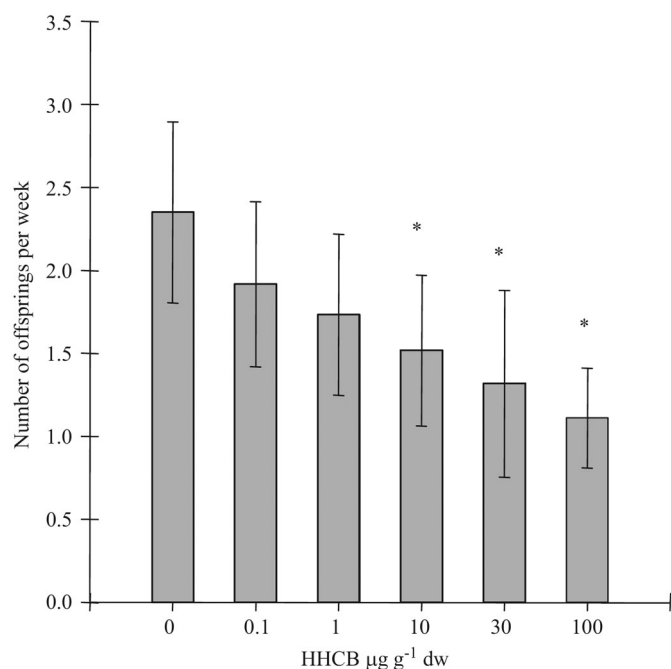
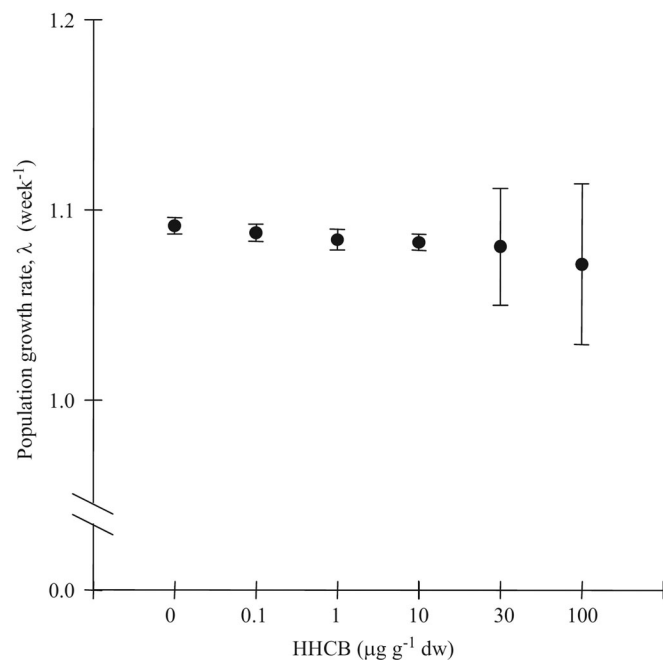**Figure 7.** Reproductive output, averaged for weeks 5–10, vs HHCB concentration.

Figure 9. Elasticity vs HHCB concentration. Squares are S_j, crosses are S_a, Triangles are t_j and circles are F.

Figure 8. Population growth rate vs HHCB concentration.

3.9. Elasticity

The elasticity analysis showed that population growth rate was several times more sensitive to relative changes in juvenile survival than to changes in the other life-history parameters (Figure 9). The life-cycle trait having the second highest elasticity was adult survival followed by time to first reproduction and fecundity, in that order. There was no notable change in the elasticity patterns among treatments.

3.10. Decomposition

The proportional contributions of fecundity (F), time to first reproduction (t_j), juvenile survival (p_j) and adult survival (S_a) to the observed effect on λ are shown in Figure 10.

Decomposition analysis showed that for all treatments the largest proportional contribution to the (non-significant) change in population growth rate (λ) was from fecundity. In snails exposed to 0.1, 1 and 10 μg HHCB g^{-1} there was a small contribution from time to first reproduction (which was positive in the 0.1 μg HHCB g^{-1} treatment because time to first reproduction was shorter). For 30 and 100 μg HHCB g^{-1}, fecundity, time to first reproduction and juvenile survival contributed to the changes in λ. As there were no effects of HHCB on adult survival, the contribution of S_a to changes in λ was zero.

4. Discussion

4.1. Sediment concentrations of HHCB

Our recoveries of HHCB are similar to those reported in other studies. Difrancesco *et al.* (2004) found soil extraction efficiency to be 87 ± 9% and leachate efficiency to be 79 ± 9%. Simonich *et al.* (2000) found a recovery of 68.2 ± 12.4% for active sludge and 85.1 ± 0.4% for influent solids.

with the highest mean value in the control group (λ = 1.092 at 0 μg HHCB g^{-1}) and the lowest in snails exposed to 100 μg HHCB g^{-1} (λ = 1.071), a difference of 1.85%. However, using the overlap of the 95% CIs around λ to estimate significance, following Sibly *et al.* (2000), the slight differences in λ among treatments were not significant. Therefore a NOEC for λ could not be estimated, but is greater than the highest exposure concentration of 100 μg HHCB g^{-1}.
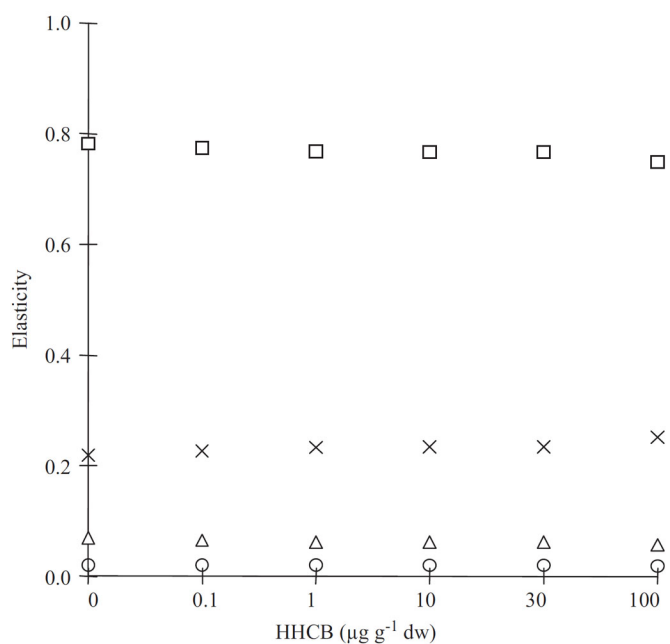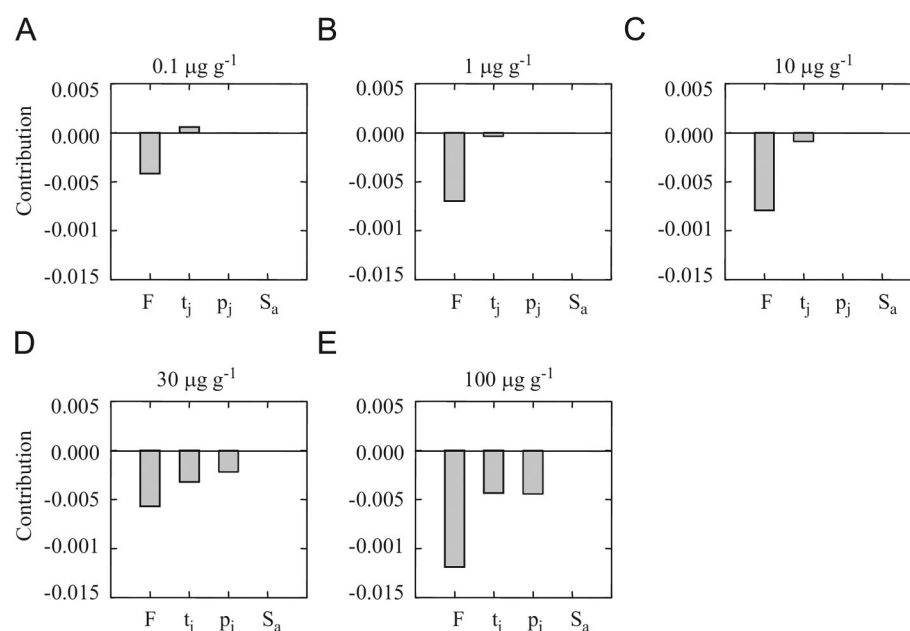
Figure 10. Proportional contributions of fecundity (F), time to first reproduction (t_j), juvenile survival (p_j) and adult survival (S_a) to the observed effect on λ at an HHCB concentration of (A) 0.1, (B) 1, (C) 10, (D) 30 and (E) 100 μg g^{-1}.

4.2. Effects of HHCB at the individual level

Adult survival was 100% in all treatments, whereas juvenile survival was reduced at 30 and 100 μg HHCB g^{-1} (by 10% and 20%, respectively). Of the remaining traits, reproduction (averaged from 5 to 10 weeks of exposure) was the most sensitive (affected at 10 μg HHCB g^{-1}) followed by adult feeding rate and juvenile growth rate (affected at 30 μg HHCB g^{-1}), and time to first reproduction (marginally affected at 30 μg HHCB g^{-1}). The least sensitive traits were adult growth rate (not affected) and size at first reproduction (marginally affected at 100 μg/g).

For the control group, average reproduction was 2.6 (± 0.6) juveniles per female per week. This value is in good agreement with data reported from other studies (Jacobsen and Forbes, 1997; Jensen *et al.*, 2001). The mean value for time to first reproduction in the control group was 39 weeks, whereas Jensen *et al.* (2001) found an average time to first reproduction of approximately 35 weeks under similar laboratory conditions. The mean value for size at first reproduction was 3.6 mm for the control group, compared to 4.2–4.7 mm reported by Jensen *et al.* (2001). The slightly slower growth in the present study is likely due to the fact that snails were maintained in the dark, whereas Jensen *et al.* (2001) maintained snails under a 12 h light:12 h dark cycle, which would have allowed growth of benthic microalgae, a preferred food source for *P. antipodarum*.

HHCB exposure significantly reduced juvenile growth at 30 and 100 μg HHCB g^{-1} but did not affect adult growth. This may be explained by either a higher sensitivity of juveniles to the chemical or by the minimal growth rate of adult snails compared to juveniles or both.

Average adult feeding rate per week was significantly reduced by HHCB exposures of 30 and 100 μg HHCB g^{-1}, and there was a significant interaction between time and exposure concentration on feeding rate, such that there appeared to be an increase in feeding rate with time for the 1, 10 and 30 μg HHCB g^{-1}, suggesting that snails may have acclimated to HHCB exposure. Feeding rate in the 100 μg HHCB g^{-1} did not increase with time and was, on average, lower than in the control group.

Table 2. Percentage differences between the control treatment and HHCB treatments for different life-history traits and λ

Treatment (μg g^{-1})	0.1	1	10	30	100
F	−17.37	−27.41	−30.50	−23.55	−42.86
t_j	0.77	−0.51	−1.28	−4.60	−6.65
S_a	0.00	0.00	0.00	0.00	0.00
p_j	0.00	0.00	0.00	−0.26	−0.53
λ	−0.33	−0.66	−0.80	−1.01	−1.85

Although adult snails in 1, 10 and 30 μg HHCB g^{-1} treatments initially showed somewhat lower feeding rates compared to the control, feeding rates returned to similar levels as those of the control group by the end of the experiment. This may suggest that snails exposed to intermediate concentrations of HHCB acclimated to the exposure after a few weeks. The reduced feeding rates during the first few weeks of the experiment may help to explain the reduction in average reproductive output during weeks 5–10 and are consistent with the observed reduction in juvenile growth rate in the two highest exposure treatments.

4.3. Effects of HHCB at the population level

Population growth rate (λ) was greater than one for all treatments, which is characteristic of a growing population, and was reduced by 1.9% at the highest exposure concentration compared to the control (Table 2). For the control group the mean population growth rate was 1.092. This is close to the mean population growth rate reported by Jensen *et al.* (2001) of approximately 1.102 for this species under similar laboratory conditions.

The increasing variability in λ at the two highest concentrations (i.e., 30 and 100 μg g^{-1}; Figure 8) is due to the fact that the contribution of the standard error in p_j to the standard error in λ (calculated as $\sqrt{[p_j(1 - p_j)/n]}$, following Sibly *et al.*, 2000) was zero for the 0, 0.1, 1, and 10 μg HHCB g^{-1} treatments (since juvenile survival was 100%), whereas for 30 and 100 μg HHCB g^{-1} there was 90%

and 80% juvenile survival, respectively, and a corresponding non-zero contribution of the standard error in p_j to the standard error in λ. Due to a relatively high demographic sensitivity of p_j, this trait contributes a great deal to the analytically calculated CI for λ (Sibly *et al.*, 2000).

Whereas fecundity was the most sensitive of the individual traits to HHCB exposure, the elasticity analysis demonstrated that this trait was the least sensitive demographically. This means that a given proportional change in fecundity will have much less of an impact on population growth rate than the same proportional change in the other life-history traits. In contrast, juvenile survival had the highest elasticity of all traits, followed by adult survival and time to first reproduction, and all three traits were less sensitive to HHCB exposure than fecundity. This is a common pattern in demographic studies (Pfister, 1998), in which it has been shown for a wide range of taxa that fecundity is generally more sensitive to environmental variability than either survival or growth, and that fecundity likewise has a lower elasticity than these other life-history traits. It has been proposed that this is the result of natural selection altering life histories to minimize life-history stages with both high demographic sensitivity and high variation since variability in population growth rate is thought to have negative consequences for organism fitness (Pfister, 1998). This may have interesting implications for the choice of toxicity test endpoints and for extrapolating these to ecologically relevant impacts.

Whereas elasticity analysis provides insight into the potential importance of changes in life-history traits for population dynamics (i.e., it asks *if* a life-history trait changes by a given proportion *then* what would be the corresponding proportional change in λ, all else being equal), decomposition analysis is critical for understanding the mechanisms underlying the observed impacts on λ in a particular environmental setting. For example, despite its high elasticity, juvenile survival made no contribution to the observed change in λ at the lowest concentrations (i.e., 0.1, 1, and 10 µg g^{-1}), a very small contribution at 30 µg HHCB g^{-1} and a slightly higher contribution at 100 µg HHCB g^{-1}. This is because juvenile survival was only minimally affected by HHCB (if it had been more affected, the impact on λ would have been substantial). Whereas fecundity had the lowest elasticity, it nevertheless made the largest proportional contribution to the (non-significant) impact on λ because it was so much more sensitive to HHCB compared to the other life-history traits.

Because of the relatively long life cycle of *P. antipodarum*, we decided to run the juvenile and adult exposures in parallel (i.e., using different individuals). This could potentially have resulted in an underestimation of effects on the adult stage. For example, we observed reduced juvenile growth rates at the highest exposure concentrations. This could have resulted in a reduced fecundity compared to individuals first exposed as adults, and/or a reduced survival of adults. However, given the low elasticities of both fecundity and adult survival, we do not believe that our design resulted in a large underestimation of population-level impacts.

4.4. Comparison of individual- and population-level effects

If we compare the percentage differences between the control treatment and HHCB treatments for each life-history trait and λ (Table 2) it is apparent that effects of HHCB on sensitive individual-level endpoints are attenuated at the population level. For all the treatments the highest percentage impacts were on fecundity, and these increased with increasing concentration (from 17% at 0.1 µg HHCB g^{-1} to 43% at 100 µg HHCB g^{-1}). There were some differences in time to first reproduction following the same pattern as fecundity. Juvenile survival (p_j) showed no difference at the lowest concentrations but a slight percentage difference at 30 and 100 µg HHCB g^{-1}. For population growth rate (λ) there were only small differences between the control treatment and HHCB treatments, but these differences increased with increasing expo-

sure concentrations (from 0.33% at 0.1 µg HHCB g^{-1} to 1.85% at 100 µg HHCB g^{-1}). Assessing likely impacts on population growth rate from impacts on single individual-level endpoints can result in underestimates or overestimates. For example, if juvenile and adult survival were the only traits measured in this case, the expected impact for the population would have been slightly underestimated. In contrast, basing the assessment on fecundity alone would substantially overestimate likely impacts on populations. A similar pattern has been observed in other laboratory experiments on other chemicals with terrestrial and aquatic invertebrates (Hansen *et al.*, 1999a,1999b; Linke-Gamenick *et al.*, 1999; Jensen *et al.*, 2001; Widarto *et al.*, 2004). But the extent to which, and under what conditions, impairments in fecundity are generally likely to overestimate impacts on population dynamics are questions in need of further attention.

4.5. Extending the analysis to ecologically realistic conditions

It is important to emphasize that the approach used here cannot (and does not intend to) make predictions about actual population sizes under different exposure conditions at some future time point. Nor does it include many additional ecological complexities that may be important in an actual field situation. Nevertheless simple models of the kind employed here provide valuable information about the robustness of a population, summarizing its capacity for future growth, and the relative importance of changes in life-history traits on population dynamics (Ferson *et al.*, 1996; Caswell, 2001). A potential criticism of the approach used in the present study is that it was conducted under favorable laboratory conditions in which snails were not subject to competition or predation and in which population growth rate was greater than one. There are concerns that under more ecologically realistic conditions populations may be more susceptible to toxic chemicals because their life-history traits are impacted by other stressors. One possible way to address such concerns is to adjust the values of the life-history traits of control snails toward more field-realistic values, to assume the same proportional impacts of HHCB on individual life-history traits, and to recalculate expected impacts on population growth rate. We did this for the present data by considering three more ecologically realistic scenarios (Table 3). In Scenario 1 we reduced survival of juvenile snails in the control group to 10% and kept all other traits unchanged. In Scenario 2, we reduced survival of juvenile snails to 10% and reduced survival of adult snails to 80%. Scenario 3 was as Scenario 2, but here we also reduced fecundity by 50% in the control group snails. Table 3 shows how λ changes for each of these scenarios, with Scenarios 2 and 3 approximating a λ of one, which is the expected long-term average for stable field populations. Next we calculated life-history trait values for each HHCB exposure concentration, assuming that the traits showed the same proportional change (i.e., were equally as sensitive to HHCB) as in our experimental treatments. Figures 11A–D show the results of elasticity analyses for each of the scenarios compared to the experimental results. This analysis shows that as the life-history traits are altered to more ecologically realistic values, their elasticity patterns

Table 3. Values for life-history traits and population growth rate measured in the laboratory control group and as they were adjusted for the three scenarios Traits that were changed relative to the laboratory control in each scenario are shown in **bold**.

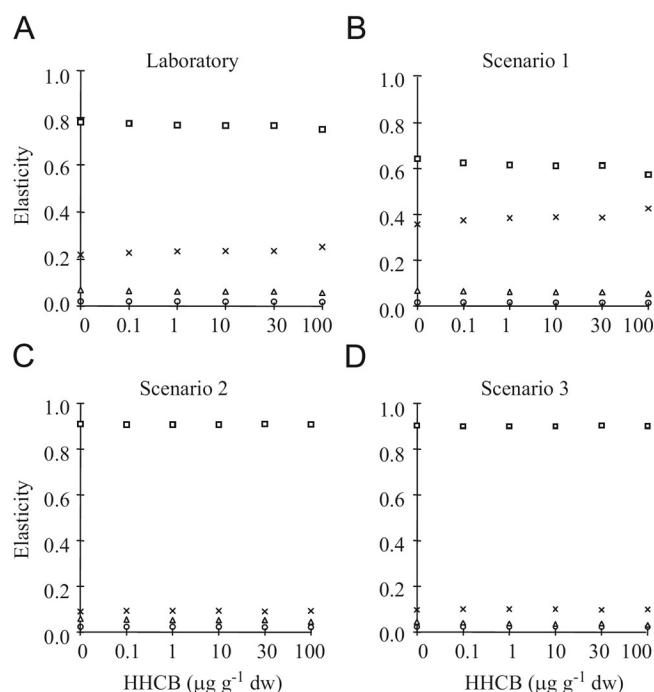| Trait in Control	Laboratory	Scenario 1	Scenario 2	Scenario 3
F	2.59	2.59	2.59	**1.30**
S_j	1	**0.1**	**0.1**	**0.1**
S_a	1	1	**0.8**	**0.8**
t_j	39.1	39.1	39.1	39.1
t_a	1	1	1	1
λ	1.0917	1.0463	1.0060	0.9901

Figure 11. Results of elasticity analyses for (A) laboratory conditions and (B)–(D) Scenarios 1–3, respectively. Squares are S_j, crosses are S_a, Triangles are t_j and circles are F.

Table 4. Calculated effects of HHCB on λ measured under laboratory conditions and in the three scenarios described in Table 3

HHCB (µg g^{-1})	Laboratory	Scenario 1	Scenario 2	Scenario 3
0	1.0917	1.0463	1.0060	0.9901
0.1	1.0881	1.0432	1.0016	0.9855
1	1.0844	1.0407	0.9986	0.9828
10	1.0830	1.0398	0.9976	0.9819
30	1.0807	1.0389	0.9974	0.9822
100	1.0714	1.0324	0.9886	0.9739
Max. % reduction	1.85	1.33	1.73	1.64

The maximum percentage reduction in λ (for the 100 µg g^{-1} treatment) is shown in the bottom row.

change. For example, when juvenile survival is reduced, keeping all else equal, its elasticity reduces and the elasticity of adult survival increases (Figure 11B). Of the most importance, however, is that in none of the scenarios is the effect of HHCB on λ greater than in the laboratory experimental system (Table 4). This suggests that population-level impacts calculated from our laboratory measurements are a fairly robust indicator of likely impacts of HHCB on populations of *P. antipodarum* under more realistic field conditions. But clearly, this scenario approach is limited in that it does not consider all potential ecological complexities that might occur in the field.

5. Conclusion

In conclusion, life-table response experiments in combination with simple demographic models can provide a powerful tool for interpreting the responses of organisms at the individual level in terms of

likely impacts on population dynamics. Our analyses showed that fecundity was the most sensitive individual-level endpoint to HHCB exposure, but that the relatively large impact on this trait was not reflected in a correspondingly large impact on population growth rate, which is in keeping with expectations based on evolutionary theory. Sediment concentrations of HHCB in the field have been reported to occur in the range of 0.002–2.2 µg g^{-1} dw sediment. Comparison of these exposure concentrations to the results of the present study suggests that populations of *P. antipodarum* are unlikely to be at risk from HHCB exposure under natural field conditions.

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