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Nonylphenol Stimulates Fecundity but not Population Growth Rate (λ) of *Folsomia candida*

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Abstract

The toxicity of nonylphenol (NP) to springtails was pronounced at 40 mg/kg dry weight soil, at which no animals survived. Body length and fecundity were the individual life-history traits significantly stimulated by sublethal concentrations of NP during a 64-day experiment. However, the effects of NP on these traits did not result in a statistically significant increase in population growth rate (λ). Decomposition analysis indicated that fecundity was the main contributor to the (non-significant) changes observed in λ . However, since the elasticity of fecundity was very low, large changes in fecundity resulted in a minimal effect on λ . Juvenile survival had the highest elasticity of all traits, but was not affected by NP, and therefore did not contribute to effects on λ . This study confirms previous studies showing that effects of chemicals on individual life-history traits are attenuated at the population level and that λ is an appropriate endpoint for ecotoxicological studies.

Keywords: elasticity, life-history, population dynamics, hormesis, *Folsomia*

1. Introduction

Ecotoxicology aims to understand and predict the effects of xenobiotics on ecological systems, i.e., on populations, communities and ecosystems. However, in practice the majority of tests used for regulatory and legislative purposes focus on individual-level responses that are relatively easy, inexpensive and rapid to measure. These advantages enable toxicity tests to be conducted for many chemicals on different species of organisms by many laboratories routinely (Laskowski, 2001). However, accurately extrapolating such test results to ecologically relevant consequences remains an important challenge.

It is well known that chemical exposure-response relationships of individual life-history traits may differ markedly from each other and from the exposure-response relationship for population growth rate. For example, the response of individual reproduction to chemical exposure

may either be more (e.g. Scott-Fordsmand et al., 1997; Van Gestel and Hensbergen, 1997; Hood et al., 2000) or less sensitive than individual growth rates (e.g. Van Straalen et al., 1989). In general, juveniles are more sensitive than adults to toxicant exposure (Stark and Banken, 1999). However, effects on juvenile survival do not always lead to reductions in population growth rate since survivors may compensate for the lost individuals by increasing their own reproduction (Walthall and Stark, 1997; Bechmann, 1999). Kam-menga et al. (1996) showed that the most toxicant-sensitive trait (the reproductive period) did not result in any significant effect on population growth rate of the nematode, *Plectus acuminatus*, whereas effects on a relatively toxicant-insensitive trait (time to first reproduction) had a large influence on population growth rate. Jensen et al. (2001) observed large reductions in the most sensitive individual life-history trait (reproductive output) after cadmium exposure, which resulted in smaller, though significant, re-

ductions in population growth rate of the snail *Potamopyrgus antipodarum*. Effects at the individual level may even be in opposite directions to those occurring at the population level. For example, despite increased growth of juvenile blowflies, *Lucilia sericata*, in response to exposure to sublethal concentrations of cadmium, population growth rate declined (Moe et al., 2001). These studies highlight the complex relationship between toxicant effects on individual performance versus population dynamics.

The present study was designed to explore the relationships between individual- and population-level responses of a terrestrial invertebrate to a toxicant. We measured the effects of nonylphenol (NP) on individual life-history traits of the springtail (*Folsomia candida*) and, using a two-stage life-cycle model (Calow and Sibly, 1990), we analyzed the effects of NP on population growth rate (λ). To determine the relative contribution of the life-history traits to the observed effects of NP on λ and to examine the (relative) demographic sensitivity of λ to changes in each of the life-history traits, we performed decomposition and elasticity analysis, respectively.

NP is a chemical contained in many industrial, household and agricultural products as NP ethoxylates. In the environment, NP will be produced again by aerobic degradation of its ethoxylates (Nimrod and Benson, 1996). Numerous studies have examined the effects of NP on many species of mostly aquatic animals and have indicated that NP is not only highly toxic (Granmo et al., 1989), but also may have endocrine disrupting properties at sublethal exposure concentrations (Yadette et al., 1999). In addition, NP is also bioaccumulated (Johnson et al., 1998) and resists degradation under anaerobic conditions (Maguire, 1999). Studies on effects of NP on soil animals are scarce despite the fact that NP may enter the terrestrial environment mainly through sludge produced in wastewater treatment plants that is subsequently used as fertilizer or (though to a lesser degree) through pesticides utilizing NP as an adjuvant.

Folsomia candida is a common, soil-dwelling, wingless, unpigmented and parthenogenetic insect that is widely distributed around the globe, abundant and plays an important role in soil ecosystems. Because of its abundance and distribution, as well as its ease of culture and short reproductive cycle, the springtail is one of the most extensively used animals in terrestrial ecotoxicology.

2. Materials and methods

2.1. Test animals

A laboratory culture of *Folsomia candida* established at the National Environmental Research Institute (NERI), Denmark was used for this experiment. In NERI, field-collected animals were maintained as stock cultures in Petri dishes filled with a substrate of moistened plaster of Paris and charcoal. They were kept at a constant temperature of 20°C and under a 12 h/12 h light/dark regime. New substrates with dried yeast as fresh food were prepared every 2-4 weeks and added *ad libitum* to the cultures. Prior to the experiment juveniles were obtained by hatching eggs collected from stock cultures. Only 0-1-day-old juveniles were employed for this experiment.

2.2. Soil preparation and application procedures

Light-textured, sandy loam agricultural soil from Askov Experimental Station, Denmark was utilized for the experiment. The soil was dried and defaunated at 80°C and sieved through 1 mm mesh. For the first 2 weeks, we used soil sieved to 250 μ m to ease measurement of the very small juveniles. According to Sverdrup et al. (2002) this same soil (though sieved to 2-mm) consisted of 38.4% coarse sand (200-2000 μ m), 23.6% fine sand (63-200 μ m), 10% coarse silt (20-63 μ m), 12.3% fine silt (2-20 μ m), and 13% clay (<2 μ m), 2.8% humus and 1.6% organic carbon, had a pH_{H₂O} of 6.2, a density of 1.135 g/cm³ dry soil and a total cation exchange capacity of 8.14 meq/100 g soil.

NP (Aldrich, Cat. no. 29.005.8, 100% pure) concentration series were prepared by diluting a stock solution in acetone (J. T. Barker, Hayward, CA, USA). The stock solution was prepared corresponding to the highest test concentration, 40 mg/kg dry weight (dw). To contaminate the soil, 225 ml solution was added and mixed thoroughly into 1.5 kg of the soil. Pure acetone was used for the control soil. The solvent was evaporated for 24 h under a fume hood. The dried contaminated soils were then put in plastic bags and stored in a deep freezer (-20°C) until use. Just prior the start of the experiment, the soil was moistened by 15% distilled water (by weight).

All effects of NP in the present study are based on nominal concentrations added to soils. Analytical measurements of these same soils were performed for a separate study with *Dendrobaena octaedra* and showed that the concentration of NP in the soil was relatively unchanged after being kept 1½ years in a deep freezer (-20°C) (Widarto et al., 2004). Weekly changing of the soils ensured that the springtails were exposed to a relatively constant concentration of NP throughout the experiment.

2.3. Experimental set-up

Nunc multi-well dishes (NUNC A/S, Roskilde, Denmark), with 24 circular wells (diameter = 1.5 mm) were used to keep individual springtails. Before new multi-well dishes were used, they were treated with destaticizer to reduce static electricity. Each well was filled with 2.5 g soil containing 15% deionized water. The soil was compressed with a pestle to obtain a firm and even surface, which aided measurement by preventing the animals from hiding in crevices. Care was taken to avoid an excessive pressure that makes the surface wet. After adding 2-3 granules of dried baker's yeast (*Saccharomyces cerevisiae*) as food in each well, a double layer of aluminum foil was used to cover all wells to prevent the springtails from escaping. To reduce water evaporation, the multi-well dishes were covered with thick cardboard glued to the original cover. All multi-well dishes were put in a closed plastic box layered with wet tissue paper at the bottom. The soils were renewed once a week during which no water was added to the substrates to minimize disturbance. When the animals started to produce eggs, only the adults were transferred into new soils. The eggs were left in the old soils until they hatched.

Five sublethal concentrations plus a control were used: 0, 8, 16, 24, 32, and 40 mg/kg dw soil after a range-finding test found that only 42% of the juveniles survived 45 mg/kg dw soil. One neonate juvenile (0-1-day-old) was added to each well of the multi-well dishes. Twelve replicates per treatment were used.

Digital image processing equipment (DIP) was used to record individual body lengths without disturbing the animals during the observation. DIP consisted of a video camera mounted on the ocular lens of a stereomicroscope that was connected to a monitor and a computer. Pictures of living specimens were recorded on the hard disk or on videotape and measured manually.

We measured body length from the posterior end of the abdomen to the anterior end of the head between the antennae (in the moving position). Body lengths were measured using the image analysis software Sigma Scan Pro (v.5, SPSS, Chicago, IL). Survival, molting,

egg production and egg development were monitored. The animals were checked every day until the end of the experiment to determine molting since this species eats its own exuviae. Newly hatched neonates were quantified by counting the number of empty eggshells.

Twelve replicates were employed for survival and body length, however, as the animals got older and some of them died, the number of replicates decreased. For reproductive endpoints, six replicate individuals were chosen randomly from the original 12 individuals.

2.4. Population growth (λ), elasticity and decomposition analysis

To calculate λ for each treatment we fit the life-history data to a two-stage model (Calow and Sibly, 1990):

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

where n is the number of hatched eggs per brood, S_j represents juvenile survival (the probability that a juvenile survives from birth to first reproduction), S_a represents adult survival (i.e. the average probability that an adult survives between census days), t_j is the average time to first reproduction, and t_a time between broods.

Elasticity analysis was carried out to determine the relative sensitivity of population growth rate (λ) to changes in each of the life-history traits contributing to it. To measure the elasticity we employed the equation: $e_a = (a/\lambda)(d\lambda/da)$, where a is a life-history trait and $d\lambda/da$ is the sensitivity of λ to changes in the particular life-history trait, a (Caswell, 2001). The elasticity was calculated based on Equations (3)–(7) in Forbes et al. (2001) and $T = nS_jt_j + S_at_at_a$. Decomposition analysis was carried out to determine how much each life-history trait contributed to the observed treatment effects on λ . The contributions of treatment effects on each life-history trait to the overall effect on λ were calculated following Caswell (1989) and Levin et al. (1996). For the elasticity and decomposition analyses, juvenile survival (S_j) is expressed on a per unit time basis in order to make this trait independent of the length of the juvenile period (t_j). For these analyses we used p_j where $S_j = p_j^{t_j}$.

2.5. Statistical analysis

Since, there were no survivors in the highest concentration (40 mg NP/kg), all statistical analyses were carried out without including this concentration. Repeated measures analysis of variance (ANOVA) was used to test for treatment effects on body weight and egg production. One-way ANOVA was used to test for treatment effects on time to first reproduction, development time and egg viability. Tukey's pairwise comparisons were performed when the ANOVA detected significant treatment effects. Effects were considered significant when $p \leq 0.05$, and considered marginally significant when $0.05 < p \leq 0.01$. All statistical analyses were performed with SYSTAT Version 10.

The effects of NP on population growth rate were analyzed by calculating 95% confidence intervals around λ for each concentration using an analytical method (Sibly et al., 2000). The calculation of the confidence intervals was performed by calculating the total variance of λ , which is the sum of the variance contributions from each of the five life-history traits contributing to λ . The square root of the total variance is the standard error of λ . Observed differences between the population growth rates were deemed significant if there was no overlap between the confidence intervals.

3. Results

3.1. Survival

Except in the highest concentration (40 mg/kg), all juveniles of *Folsomia candida* survived until first reproduction. In the highest concentration, springtails survived for only

2–6 days. During the adult period, NP showed no clear concentration-response effect on survival. Excluding the highest concentration, survival was marginally impaired by NP ($p = 0.071$), but Tukey's pairwise comparisons showed no differences between any two concentrations ($p > 0.05$).

3.2. Growth

Body length of *F. candida* increased with time following a sigmoid growth curve. During the 64-day experiment, body length increased significantly from an average of 0.40 to 2.10 mm (effect of time $p < 0.01$) with maximum length attained at 30–34 days of age (Figure 1). NP also affected body length ($p < 0.01$) and the interaction between time and NP was significant ($p = 0.002$).

NP stimulated growth at the lower concentrations (i.e., up to 24 mg/kg) and at 32 mg NP/kg, body size was less than the control only for days 20 and 30 (Figure 1). After 30 days of age, when the body length of springtails reached between 2.04 mm (the lowest) in the control and 2.20 mm (the highest) in the 16 mg/kg concentration, effects of NP were not detectable.

3.3. Molting

F. candida released their first exuviae (cuticle) at the age of 3–4 days. NP reduced the time to first molt ($p = 0.01$). Tukey's pairwise comparison test showed that time to first molt in 16 mg/kg ($p = 0.027$) was significantly shorter than in the control. Marginally significant differences were observed between the control and 24 mg/kg ($p = 0.079$) and between 16 and 32 mg/kg ($p = 0.063$). In the highest concentration (40 mg/kg), only two of 12 individuals molted. After the first exuviae, *F. candida* cast off its cuticle every 3–4 days, and NP did not affect this molting period ($p = 0.12$). Thus, during the 64-day experiment, 16 moltings were recorded on average.

Figure 1. The growth of *Folsomia candida* on Askov sandy loam soil, expressed as body length (mean $\pm$ SEM) during 64 days of exposure to nonylphenol.

Figure 2. (a) Mean number of eggs of *Folsomia candida* on Askov sandy loam soil produced per individual per brood during 64 days of exposure to nonylphenol. Error bars = SEM. (b) Mean number of eggs of *Folsomia candida* on Askov sandy loam soil per individual produced during 64 days of exposure to nonylphenol (open circles represent individual data; filled circles represent means $\pm$ SEM).

3.4. Reproduction

Springtails reproduced for the first time at an age of 15–16 days (Figure 2a), and we did not detect any effects of NP on this trait ($p = 0.87$). The springtails then produced eggs every 7–8 days, and no effect of NP was detected on the time between broods ($p = 0.98$). During the 64-day experiment, *F. candida* produced an average of 6–7 broods per individual.

A total of six broods was analyzed from each of six females per treatment. Whereas the early broods contained an average of 20–25 eggs per individual, brood size increased markedly with age ($p < 0.001$) reaching approximately 150 eggs per brood in the sixth brood. The aver-

age number of eggs per individual, pooled over the six broods, (Figure 2b) showed that NP significantly affected egg production in the springtails ($p = 0.006$). Tukey's pairwise comparison indicated that individuals treated with 8 and 16 mg NP/kg produced a significantly larger number of eggs than those in the control ($p = 0.031$ and 0.007 , respectively), whereas the two higher concentrations produced similar numbers of eggs as the control ($p = 0.33$ in 24 mg NP/kg and $p = 0.761$ in 32 mg NP/kg).

Separate ANOVAs were carried out to examine the effect of NP on each brood, and the results showed that NP had significant or marginally significant effects on the average number of eggs produced per individual in the first four, but not the last two broods ($p = 0.021, 0.07, 0.008, 0.01, 0.38, 0.36$, in order from the first to the sixth brood). Springtails in the 16 mg NP/kg treatment generally produced more eggs than those in the other concentrations, including the control. None of the broods produced by the highest treatment group (32 mg NP/kg) differed from the control group ($p > 0.05$ for all comparisons).

3.5. Egg viability and development time

We tested the first five broods produced for effects of NP on egg viability and detected a significant effect only in the second brood ($p = 0.69, <0.01, 0.54, 0.08, 0.91$, respectively). When we pooled the data from each brood, the effect was not significant ($p = 0.68$). Most eggs (average $>95\%$) hatched in all treatments, and one batch of eggs in the 16 mg/kg had a hatching success of 100%. The lowest hatching success was 80.5% recorded from a control brood.

Development time was calculated from hatched eggs (eggs that failed to hatch were excluded). Eggs hatched after an average of 9–10 days. ANOVAs performed on the individual broods showed that NP did not have any effects on egg development time ($p > 0.05$). When pooled data were analyzed and Tukey's pairwise comparisons performed, a significant difference was only detected between 8 and 24 mg/kg ($p = 0.023$).

3.6. Population growth rate (λ), decomposition and elasticity analysis

All λ 's in the control and the NP treatments were greater than one (Figure 3). We observed small increases in λ as the NP concentrations increased up to 16 mg/kg followed by decreases at higher concentrations. λ increased by 0.46% (at 8 mg NP/kg) and by 1.55% (at 16 mg NP/kg) (Table 1). The increase in λ in 16 mg/kg resulted mainly from the positive response of fecundity that contributed 98% to the effect on λ . Based on the calculation of 95% confidence intervals, however, λ was not significantly different between the concentrations.

Since 100% of the juveniles survived in all treatments, juvenile survival did not make any contribution to the (non-significant) effect on λ . Decomposition analysis of the two-stage model revealed that the effect of NP on λ was mostly attributable to the effect on fecundity (between 61% and 98%) whereas the effect on time to first reproduction

Figure 3. Population growth rate (λ) of *Folsomia candida* on Askov sandy loam soil exposed to nonylphenol. Error bars represent 95% confidence intervals.

contributed between 0% and 35% to the total change in λ (Table 1). Time between broods contributed between 2% and 12%, and adult survival contributed very little to the change in λ since nearly all adults survived.

Figure 4 shows the relative sensitivity (elasticity) of population growth rate to changes in each life-history trait of *F. candida*. For this life cycle, λ was the most sensitive to changes in juvenile survival probability (S_j) and insensitive to changes in adult survival probability (S_a) (i.e., elasticity of S_a was only one hundredth of that of S_j). The relative sensitivity of λ to changes in time to first reproduction (t_j) was about one third that of juvenile survival (S_j). The relative sensitivity of λ to changes in fecundity n and time between broods (t_a) was ten- and two-fold higher, respectively, than to changes in adult survival probability (S_a). Exposure to different concentrations of NP did not change the elasticity patterns among traits.

4. Discussion

4.1. Linking individual-to population-level responses

This study showed that individual life-history traits of *F. candida* responded differently to exposure to NP and that significant stimulatory effects of a chemical at the individual level do not necessarily result in significant effects at

Figure 4. Elasticity of time to first reproduction (t_j), egg production (n), juvenile survival (S_j), adult survival (S_a), and time between broods (t_a) of *Folsomia candida* on Askov sandy loam soil at different nonylphenol concentrations. Note that to aid comparison these are values of elasticity, since increases in t_j and t_a have a negative effect on λ whereas increases in n , S_j and S_a have a positive effect on λ .

the population level. At the highest exposure concentration (40 mg/kg dw soil), no juveniles survived the treatment, and therefore none of the other traits could be assessed. At sublethal concentrations (up to 32 mg/kg dw soil), traits such as growth, time to first molting and fecundity were stimulated by NP exposure, whereas no effects of NP were observed on time between molts, time to first reproduction, time between broods, number of broods, egg viability or egg development time. Integrating the individual-level effects via a two-stage life-cycle model indicated that the overall effect of NP on population growth rate, λ , was not statistically significant and suggests that effects at the individual level are attenuated at the population level.

By using elasticity analysis we were able to explore the sensitivity of λ to changes in the life-cycle traits contributing to it (Caswell, 1989). With this analysis we estimated the proportional change in λ resulting from a proportional change in a given life-history trait, holding all other traits constant. Under the laboratory conditions used in the present study juvenile survival had the highest elasticity followed by time to first reproduction. Thus a given impact

Table 1. Percent changes of individual traits and population growth rate (λ) and proportional contribution of individual traits (%) to changes in population growth rate (λ) of *Folsomia candida* on Askov sandy loam soil, based on Caswel (1996) and Levin et al. (1996)

Concentration (mg NP/kg dw)	% changes of					Contribution (%)			
	λ	t_j	N	t_a	S_a	t_j	N	t_a	S_a
8	0.46	-2.53	22.34	-1.97	-7.5	35.00	61.41	1.33	2.26
16	1.55	0	27.59	0.56	2.27	0	98.57	0.53	0.90
24	1.24	1.04	15.99	0.18	-1.27	24.31	74.85	0.21	0.63
32	0.71	0.33	10.18	0.49	-0.04	13.28	85.66	1.02	0.04

on juvenile survival probability and time to first reproduction would be expected to have much greater consequences for population dynamics than would a similar proportional impact on adult survival, fecundity or time between broods. Whereas juvenile survival had a very high elasticity; adult survival had the lowest elasticity of all traits. However, since neither of these traits was influenced by NP (below the highest treatment concentration) they did not contribute to changes in population growth rate. In contrast, fecundity was sensitive to NP exposure, but because of its low elasticity, the consequences of changes in fecundity for λ were minimal.

Similar effects of NP have been observed in the polychaete, *Capitella* sp. I (Hansen et al., 1999) in which λ was not significantly affected although a stimulatory effect on brood size was observed. Several other studies have also indicated a lower sensitivity of λ compared to individual-level endpoints such as growth and reproduction (e.g. Stark et al., 1997). Widarto et al. (2004) found negative effects of NP on fecundity of the earthworm *D. octaedra*, but the resulting decline in λ was not significant. Bechmann (1999) did not detect an effect of NP on λ of the marine copepod *Tisbe battagliai* even though only 6% of the juveniles reached maturity at 62 μg NP/l. Apparently, the survivors were able to produce enough offspring to increase the size of the population compared to the parent generation. Walthall and Stark (1997) suggested that species with high reproductive rates may have an ability to compensate for the loss of individuals caused by toxicants such that effects on λ are minimized or nullified.

Both Kammenga et al. (1996) and Moe et al. (2001) demonstrated that reductions in population growth rate are not determined by the most sensitive life-cycle traits. Klok and de Roos (1996) suggested that the relationships between effects at the individual- and population-levels are not always straightforward and are sometimes counterintuitive. These results highlight the difficulty of using single life-history traits as simple proxies for population-level effects.

4.2. Interspecies differences in sensitivity

Negative effects of NP on various life-history traits at sublethal levels have been recorded in many studies with a variety of taxa; however there do not seem to be any general patterns among species as to which traits are the most/least sensitive. Some studies observed negative effects on growth and development but not on other traits (Widarto et al., 2004); other studies observed effects on reproduction only (e.g. Hood et al., 2000; Bettinetti et al., 2002), whereas effects of NP on other measured life-history traits were not detected. Exposure to sublethal concentrations of NP did not influence *Chironomus riparius* emergence success (Bettinetti and Provini, 2002), *Corophium volutator* sex ratio (Brown et al., 1999), various life-history traits of *Tisbe battagliai* (Bechmann, 1999), growth or reproduction in *Chironomus tentans* (emergence,

sex ratio, fecundity, and egg viability) (Kahl et al., 1997), or fecundity or sex ratio in *Daphnia magna* (Baldwin et al., 1997).

The toxicity of NP in the present study (juvenile LC50 between 32 and 40 mg/kg) was equal to or slightly higher than that observed in other similar studies (such as Madsen et al., 1998; Gejlsberg et al., 2001; Krogh et al., 1997). Several factors may have increased the toxicity observed in the present study compared to earlier work. Our study was initiated with early-stage juveniles (1-2 days old) whereas the other studies generally employed very late-stage juveniles or subadults (10-12 days old that will mature within 5-3 days), which is a standard test procedure (Riepert and Kula, 1996). Young juveniles are very active metabolically and physiologically since they are in progressive development and differentiation as demonstrated by Holmstrup and Krogh (1996) with *F. fimetaria*. In addition, younger animals generally have smaller body sizes than older individuals, and thus a higher surface-to-volume ratio resulting in more rapid rates of chemical uptake. Effects of body size may contribute to differences in sensitivity both within and among species (cf. Widarto et al., 2004; Gejlsberg et al., 2001).

A second factor contributing to differences in organism sensitivity among studies is the soil organic content. As a hydrophobic chemical, NP tends to bind to organic material in the soil so that its concentration in porewater may decrease, thereby reducing its bioavailability. The application of NP to soil via sludge is likely to increase soil organic content and thereby reduce the availability and toxicity of NP. The addition of sludge to soil (sludge:soil ratio, 1:20) increased the LC50 by a factor of 1.5 (Gejlsberg et al., 2001). In contrast, Scott-Fordsmand and Krogh (2003) found that adding NP-contaminated sewage sludge into soil did not significantly reduce the toxicity of NP to *F. fimetaria*.

4.3. Why might NP show non-monotonic concentration-response relationships?

4.3.1. Endocrine disruption

In the present study, clear stimulatory responses to NP were seen for growth and fecundity at concentrations a factor of 2.5 below those leading to complete juvenile mortality. Stimulatory effects of NP on growth and reproduction at low concentrations may be associated with the estrogenic characteristics that have been reported for this chemical. Studies of vertebrates have suggested (e.g. Yadetie et al., 1999) that NP imitates natural (endogenous) estrogen (E2), binds to and activates the estrogen receptor (ER) leading to estrogenic effects. Stimulation of certain life-history traits is likely to occur after exposure to a xenoestrogen since E2 normally stimulates tissue growth by promoting cell proliferation and enlargement in addition to initiating protein synthesis (e.g. Colerangle and Roy, 1996). In insects, juvenoids are juvenile hormones (JH) responsible for growth and the metamorphosis of juveniles into adults (Le

Blanc et al., 1999). Their action is mediated by specific receptors, and they may be susceptible to binding by xenoestrogens (WHO/IPCS, 2002).

Stimulatory effects of NP on animals have been observed at subcellular, cellular and organ levels (e.g. Hemmer et al., 2001; Yokota et al., 2001). Stimulation of animal life-history traits (especially growth and fecundity) has been detected in at least three studies (Hansen et al., 1999; Hoss et al., 2002; Duft et al., 2003). In a field study, Krogh et al. (1996) detected an increased number of *Collembola* in the presence of sewage sludge contaminated with 150 mg NP/ kg dw soil, particularly in the lower layer (10-20 cm in depth).

A common response of animals encountering xenoestrogens is an increase in vitellogenin production. All egg-laying animals, including invertebrates and insects, produce vitellogenin needed to initiate the creation of yolk protein (Byrne et al., 1989). Hill et al. (2003) reported that NP caused vitellogenin induction, delayed sexual maturation, caused production of a small percentage of ova-testes, led to a female biased sex ratio, and impaired reproductive capacity of zebra fish (*Danio rerio*). Vitellogenin production and other developmental processes, molting, embryo development, and ovulation are regulated by ecdysteroids. As for juvenoids, NP may have bound to and activated the ecdysteroid specific receptor eliciting enhanced effects on reproduction.

4.3.2. Hormesis

A second explanation for the stimulatory effects of NP is hormesis. Stebbing (1982) defined hormesis as a stimulatory response after low-dose exposure followed by inhibition at higher doses, considered to result from overcompensation to changes in homeostasis caused by stress. He suggested that the phenomenon, especially hormetic growth, represents an adaptation of the growth control mechanism due to overcorrection by the organism in overcoming the stress (Stebbing, 1998). Calabrese and Baldwin (2001) concluded that most of the hormetic dose-response relationships reported in the literature support the idea of overcompensation stimulation following an initial period of disruption instead of a direct stimulation. The period of disruption at the beginning of exposure is a critical factor in judging hormetic cases.

Calabrese and Baldwin (1998) suggested that not all stimulatory effects can be considered as hormesis, and they proposed some criteria that can be used to confirm the occurrence of hormesis, such as number of doses below the no observed effects level (NOEL), whether NOEL is determined or not, number of doses that are statistically significant, and the magnitude of the response. We observed that with five doses below NOEL, two doses were significantly different from the control; stimulation was around 11-33% and 10-27% greater than the control for growth and fecundity, respectively. In relation to the criteria proposed by Calabrese and Baldwin (1998) we propose that the stimulatory effect observed in our study can be categorized as a low to moderate hormesis. We note that stimu-

lation of growth in our study first became apparent after about 10 days of exposure, and that after 30 days of exposure no differences in body size between groups were detectable (Figure 1). Likewise, of six broods recorded, stimulation of fecundity was detectable for the first four broods, but was not significantly different from control for the last two broods. These results may indicate that the exposed collembolans acclimated to NP exposure during the course of the experiment, after being slightly affected at the beginning of the exposure period.

Whether stimulation of growth (or other life-history traits) indicates that a chemical acts as a disruptor of specific hormonal processes or whether it results from general overcompensation to low levels of stress can be difficult to determine in practice yet may have importance in interpreting the effects of chemicals on organisms. However, of most importance for ecological risk assessment is the extent to which such effects on life-history traits—whatever their underlying biochemical mechanism—result in effects at the population level.

5. Conclusions

In conclusion, the presence of NP in soil stimulated body growth rate and fecundity of the parthenogenetic springtail, *F. candida*, but did not affect population growth rate, λ . Effects of NP on fecundity contributed 80% of the observed effect on λ . However, due to the low elasticity of λ to changes in fecundity, changes in this life-history trait did not result in a significant effect on λ . The present study demonstrates the kinds of insights that can be gained by integrating effects of toxicants at the individual level to effects on population growth rate, particularly when life-history traits exhibit variable and nonlinear concentration-response curves. We emphasize that this study was conducted under controlled laboratory conditions, and other factors, such as density-dependent interactions, predators, initial population structure, habitat features, etc., may have a critical influence on the dynamics of field populations and need to be considered when assessing the likely effects of toxic chemicals in nature.

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