Variation in the response of the invasive species *Potamopyrgus antipodarum* (Smith) to natural (cyanobacterial toxin) and anthropogenic (herbicide atrazine) stressors

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Received 28 October 2004; accepted 21 February 2005

At concentrations relevant to the field, cyanobacterial toxins (natural) and atrazine (anthropogenic) are detrimental to the gastropod *Potamopyrgus antipodarum*, with a greater toxicity for the natural (vs anthropogenic) stressor.

Abstract

In the context of increasing freshwater pollution, the impact on life-traits (survival, growth and fecundity) and locomotion of *Potamopyrgus antipodarum* of a 5-week field-concentration exposure to the cyanobacterial toxin microcystin-LR and the triazine herbicide, atrazine was studied. Whatever the age of exposed snails (juveniles, subadults, adults), microcystin-LR induced a decrease in survival, growth and fecundity but had no effect on locomotion. Atrazine induced a decrease in locomotory activity but had no significant effect on the life-traits. These results are discussed in terms of consequences to field populations. © 2005 Elsevier Ltd. All rights reserved.

Keywords: *Potamopyrgus antipodarum*; Cyanobacteria; Microcystin-LR; Atrazine; Life-traits; Locomotion; Age

1. Introduction

Freshwater ecosystems are increasingly exposed to various natural and anthropogenic contaminants. Cyanobacterial toxins and the triazine herbicide, atrazine are among the most frequent contaminants in natural surface waters as a result of, respectively, growing eutrophication (Lindholm et al., 1989; Codd, 2000) and agricultural practices (in particular in North America and Europe) (Eisler, 1989; Hofman and Winkler, 1990). Due to their generally wide distribution and abundance, close association with benthic sediments, and relatively sedentary nature, molluscs are potentially ideal indicator species of water contamination (Salanki, 2000; Downs et al., 2001; Lefcort et al., 2002). As an introduced and invasive species, the prosobranch *Potamopyrgus antipodarum* (Smith) provides an interesting model among the molluscs. Originally an inhabitant of fresh waters, this species is native to New Zealand. It differs from other Hydrobiidae in being parthenogenic and ovoviviparous. It has successfully invaded Europe (Robson, 1923), Australia (Ponder, 1988), and recently North America (Bowler, 1991; Zaranko et al., 1997), migrating from brackish to fresh waters. The impact of some xenobiotics on *P. antipodarum* has been studied and it has been observed that the degree of tolerance depends on the type of stressors. Thus *P. antipodarum* is highly sensitive to heavy metals,
fungicide Triphenyltin (TPT) and anti-fouling Tributyltin (TBT) but resistant to thermal effluents and pollution linked to industrial activity (coal mining, iron) (Watton and Hawkes, 1984; Serafinski et al., 1993; Quinn et al., 1994; Jensen et al., 2001; Duft et al., 2003). Even though atrazine and cyanotoxins are among the most frequent contaminants in fresh waters, their impact on P. antipodarum does not appear to have been investigated. However, some authors have demonstrated toxic effects of atrazine on pulmonates (Streit and Peter, 1978; Baturo and Lagadic, 1996; Russo and Lagadic, 2000, 2004), and cyanobacterial toxins are known to affect benthic macroinvertebrates (Michel et al., 1972; Wear and Gardner, 2001).

Most studies have so far focused on the acute toxicity of chemicals i.e. short-term effects (usually death) of high pollutant concentrations. In natural habitats, aquatic organisms are generally exposed to relatively low concentrations of toxic pollutants for long periods. In the field, the concentration of atrazine can reach 90 μg/l but does not usually exceed 20 μg/l (Huber, 1993; Solomon et al., 1996). Microcystin-LR is one of the most commonly occurring and highly hepatotoxic cyanotoxins. Depending on environmental conditions and seasons, its concentration varies from 0 to 140 μg/l (Lindholm et al., 1989; Zurawell et al., 1999; Hyenstrand et al., 2003), and up to 1300–1800 μg/l following algicide treatment (Jones and Orr, 1994). Since the parthenogenetic forms of P. antipodarum reproduce throughout the year (Hughes, 1996 for review), all the development stages (juveniles, subadults, adults) may be exposed to the pollutants in the field, and their responses may also differ in relation to their maturity and their resource allocation patterns. In this study, we hypothesize that natural or anthropogenic pollutants can affect organisms differently, depending on the stressor and the life stage of P. antipodarum. We set the pollutant concentrations in our experiments to be equivalent to those commonly observed in European fresh waters (10 and 50 μg/l of atrazine, and 33 μg/l of microcystin-LR).

2. Material and methods section

Snails native to a population of parthenogenetic females in a wetland stream (Pleine-Fougères, France, North latitude 48°24’; West longitude 1°45’) were mass-reared under laboratory conditions (20 °C, LD: 12–12, fed on lettuce ad libitum) in 301 aquaria containing dechlorinated tap water. Before exposure to the stressors of both microcystin-LR (MC-LR) and atrazine, snails were acclimatized individually for 7 days in 75 ml glass beakers (20 °C, LD: 12–12, fed on lettuce ad libitum).

For the microcystin-LR (MC-LR) experiment, shell height at the time of exposure was 1.32 ± 0.07 mm, 2.49 ± 0.11 mm and 4.07 ± 0.14 mm, respectively, for juveniles, subadults and adults (90 contaminated and 90 control). For contamination, snails (30 juveniles, 30 subadults and 30 adults) were individually exposed for 5 weeks to 33 μg/l microcystin-LR (from the “Alexis Corporation”), renewed every week. Methanol (1 ml/l) was used to solubilize microcystin-LR. “MC-LR controls” (30 juveniles, 30 subadults and 30 adults) were maintained in tap water with 1 ml/l methanol, renewed every week.

For the atrazine experiment, shell height at the time of exposure was 1.30 ± 0.17 mm, 2.53 ± 0.18 mm and 3.55 ± 0.18 mm, respectively, for juveniles, subadults and adults (180 contaminated and 90 control). For contamination, snails were individually exposed for 5 weeks to 10 or 50 μg/l atrazine, renewed every week. “Atrazine controls” were maintained in dechlorinated tap water renewed every week.

For all the snails, size was measured to the nearest 0.1 mm every 7 days. After 5 weeks, locomotion was estimated by measuring the distance moved individually by the snails over an allowed time. Each snail was placed for 15 min in a box filled with 75 ml water from the beaker in which they had been maintained. After this period and following the removal of the snails, 10 mg of carmine were added to each box to adhere to the mucus tracks (Calow, 1974). The length of the mucus trails produced, revealed as red bands, was then measured with the help of a digital curvimeter to the nearest 1 mm (distance moved per 15 min). Then, all the snails were dissected to count the number of embryos harboured in the oviduct pouch.

Two-way analyses of variance (ANOVA) with repeated measures were performed to compare the growth of control and exposed snails during the 5-week study. Multiple comparison tests (Scheffé and Fisher’s PLSD) were performed when there was a significant interaction between time and stressor exposure. For both exposed and control snails, χ² tests were used to compare the survival rate and the proportion of gravid females. Two-way analyses of variance (ANOVA) were used for statistical comparisons of locomotion and fecundity between exposed and control groups. Spearman’s correlation coefficient, R, was calculated to investigate correlation between shell height and embryo number, and between shell height and locomotion. Differences were considered to be statistically significant at P < 0.05. Data are reported as means ± standard deviation.

3. Results

3.1. Effects of microcystin-LR exposure

Whatever the age, life-traits of MC-LR exposed snails (survival, growth, fecundity) were affected compared with controls. Locomotion was unchanged.
According to the χ² tests, the survival rate was reduced when exposed snails were young (df = 1, χ² = 29.87, 13.89 and 5.44, and P = 0.0001, 0.0002 and 0.0196, respectively, for juveniles, subadults and adults) (Fig. 1). The repeated measures ANOVA revealed no significant effect of MC-LR on growth, but a significant time-effect (F = 208, P = 0.0001 for juveniles; F = 244, P = 0.0001 for subadults; F = 63, P = 0.0001 for adults) and a significant interaction between time and MC-LR (F = 3, P = 0.009 for juveniles; F = 2, P = 0.05 for subadults; F = 4, P = 0.007 for adults) occurred (Fig. 2). The number of embryos in the oviduct pouch was significantly reduced in MC-LR exposed snails (1.30 ± 0.85 embryos) compared to the controls (2.52 ± 1.31) (ANOVA: F = 4, P = 0.049), and the proportion of gravid females was significantly different between contaminated samples (45.28%) and controls (56.90%) (df = 1, χ² = 3.927, P = 0.0475). The number of embryos (E) was proportional to the size of the females both in snails exposed to MC-LR (R = 0.58, E = 5.91Size/C = 1.98) and those which were not exposed (R = 0.51, E = 9.62Size/C = 3.13) (Fig. 3). Whatever the age, locomotion of exposed snails was not significantly different from controls (ANOVA: F = 1.47, P = 0.231 for juveniles; F = 2.93, P = 0.093 for subadults; F = 0.04, P = 0.848 for adults). Whether exposed to MC-LR or not, distance moved by the snails (D in mm/min), was proportional to their size (R = 0.31, P = 0.0001, D = 2.36Size/C = 0.49).

### 3.2. Effects of atrazine exposure

Whatever the age and the atrazine concentration (10 or 50 μg/l), the life-traits of exposed snails were not affected: the survival rate did not vary significantly (df = 1, χ² = 2.895 and 052, P = 0.0889 and 0.8197, respectively, for 10 and 50 μg/l atrazine). The repeated measures ANOVA revealed a significant time-effect on growth (F = 67, P = 0.0001 for juveniles; F = 67, P = 0.0001 for subadults; F = 113, P = 0.0001 for adults). The number of embryos (7.49 ± 2.39 for controls, 7.53 ± 2.61 with 10 μg/l atrazine and 9.65 ± 2.96 with 50 μg/l atrazine; ANOVA: F = 1, P = 0.2527) and the proportion of gravid females (80.00% for controls, 71.11% with 10 μg/l atrazine and 88.89% with 50 μg/l atrazine; df = 1, χ² = 1.731 for 10 μg/l (P = 0.1883) and 3.6 for 50 μg/l (P = 0.0578) atrazine) were unchanged. The number of embryos was pro-

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Fig. 1. Survival rate of control (−) and *P. antipodarum* exposed to microcystin-LR (33 μg/l) (+) as juveniles (J), subadults (S) and adults (A).

Fig. 2. Growth of control *P. antipodarum* (white square) and of snails exposed to microcystin-LR (33 μg/l) (black square): (A) juveniles, (B) subadults, (C) adults.
received 5 weeks of exposure to microcystin-LR (33 μg/l). Locomotion of subadult and adult snails exposed to atrazine was significantly reduced compared to the controls (ANOVA: $F = 5.558$, $P = 0.0083$ for subadults, $F = 3.615$, $P = 0.0301$ for adults), but the decrease was not significant in the case of juveniles (ANOVA: $F = 0.229$, $P = 0.796$). The reduction of locomotor activity was proportional to the atrazine dose whatever the age: $14.17 \pm 5.94$ mm/min for the controls, $12.47 \pm 4.80$ mm/min with 10 μg/l and $11.49 \pm 4.69$ with 50 μg/l (ANOVA: $F = 5.055$, $P = 0.0072$). Whether exposed to atrazine or not, the distance moved by the snails, was proportional to their size ($R = 0.42$, $P = 0.0007$ for controls, $R = 0.37$, $P = 0.0048$ for 10 μg/l atrazine and $R = 0.46$, $P = 0.0002$ for 50 μg/l atrazine) (Fig. 4). Relationships between the distance moved ($D$ in mm/min) and the size of the snails were: $D = 3.21$Size + 4.49 for controls, $D = 3.30$Size + 3.79 for snails exposed to 10 μg/l atrazine, and $D = 2.40$Size + 4.18 for snails exposed to 50 μg/l atrazine.

4. Discussion

Pollutants affect organisms differently depending on the nature of the stress. Though toxicity of MC-LR is more acute for juveniles, life stage (juvenile, subadult, adult) is less significant. The herbicide triazine, atrazine is a non-polar pesticide, inhibitor of photosynthesis, and the hepatotoxic is a potent inhibitor of protein phosphatases (type 1 and type 2A) which are involved in homeostasis. MC-LR is also a potent tumour promoter. Whatever the age of the snails, atrazine exposure induced a decrease in locomotor activity of P. antipodarum without affecting the life-traits, whereas MC-LR exposure severely affected survival, growth and fecundity without having an effect on locomotion. In particular, the number of embryos harboured by females was halved on exposure to MC-LR. Compared to atrazine, MC-LR appears greatly more toxic for P. antipodarum. These various responses to stressors such as atrazine and MC-LR may characterize this clone of P. antipodarum. European populations of P. antipodarum appear to be strictly parthenogenetic and the three morphologically separable strains recognized in Britain are each monoclonal (Hughes, 1996). Atrazine and MC-LR may have different effects on individuals originating in either monoclonal or bisexual populations. Depending on the stressor and its degree of toxicity, the consequences of atrazine and MC-LR are expected to be different for the populations in the field. Thus one can hypothesize a negative impact of MC-LR on P. antipodarum inland populations, based on the strong decrease in embryo production and survival induced by MC-LR exposure. In this context, toxic cyanobacteria may be considered as a potential ecological factor in the structuring of freshwater gastropod communities. Bio-accumulation of both atrazine (Munoz and Roses, 2000) and MC-LR (Zurawell et al., 1999) has been demonstrated in pulmonates, involving a risk of contaminant transfer through the food web. The impact of cyanobacteria and their toxins on the life-traits of snails has been little investigated. Gevrey et al. (1972) showed no change in the survival of the pulmonate Radix auricularia after ingestion of cyanobacteria, but showed a strong acute toxicity of their cyanotoxins after direct exposure (death following motor disorders). On the contrary, consequences of atrazine exposure on snails are well documented. There is good evidence for susceptibility of gastropods to atrazine at levels inferior to 20 μg/l. Examples include Ancylus fluviatilis, which showed decreased survival, egg production and hatching rate and increased activity and ingestion (Streit and Peter, 1978). A. fluviatilis and Physa acuta which showed
changes in grazer behaviour, increased searching velocity and different movement patterns (Roses et al., 1999), *Lymnaea stagnalis* which showed a significant modulation of immune function (Russo and Lagadic, 2004), *Lymnaea palastris* which showed an immunological reaction resembling a trematode parasitism (Russo and Lagadic, 2000) and an inhibition of enzyme activities (Baturou and Lagadic, 1996) without changes in life-traits and glycogen metabolism (Baturou et al., 1995). Effects have been documented for contaminant concentrations of up to 50 μg/l of atrazine (Huber, 1993; Solomon et al., 1996 for reviews).

These findings suggest that firstly, the response of *P. antipodarum* depends on the contaminant and secondly, there are species-specific differences in response to these contaminants amongst gastropods. They also show the complexity of the field situation, due to the co-occurrence of numerous contaminants of different origins (natural and anthropogenic) in freshwater ecosystems, and the potential amplification of toxic effects. In this context, to analyse and to predict the impact of natural (cyanobacterial toxin) and anthropogenic (herbicide atrazine) stressors on both gastropod population dynamics and the whole ecosystem are difficult. However, we note that *P. antipodarum* is considered to be an invasive species in the continents where it has been introduced. The sensitivity of *P. antipodarum* to pollutants may limit its potential for further invasion and could lead to local extinctions of populations.

**Acknowledgments**

We are grateful to Georges B. J. Dussart for critical review and improvements in the English.

**References**


