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Exposure of the snail *Potamopyrgus antipodarum* to herbicide boosts output and survival of parasite infective stages ☆

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ABSTRACT

Anthropogenic stressors such as pollutants can modulate levels of parasitic infections in aquatic animals by suppressing host immunity or through some other mechanisms. One such mechanism could involve increases in either the quantity or quality of infective stages produced by parasites. We investigated the effect of exposure of infected snails, *Potamopyrgus antipodarum*, to different concentrations of the widely-used herbicide glyphosate, on (i) the production of infective cercariae by three trematode species, *Coitocaecum parvum*, *Apatemon* sp. and an undescribed renicolid, and (ii) the survival of cercariae of the latter species. For all three trematode species, infected snails exposed over a month to low (0.36 mg a.i. L^{-1}) or medium (3.6 mg a.i. L^{-1}) formulated glyphosate concentrations released between 1.5 and 3 times more cercariae per day than snails under control conditions. The similar pattern seen in all trematodes suggests a general weakening of the host benefiting any of its parasites rather than some parasite species-specific mechanism. In addition, the survival of renicolid cercariae improved with increasing glyphosate concentrations, with cercariae living about 50% longer in the medium concentration (3.6 mg a.i. L^{-1}) than in control conditions. Our results demonstrate a clear interaction between glyphosate pollution and parasitism by trematodes in freshwater systems, occurring at glyphosate concentrations recorded in aquatic habitats, and within the environmental exposure limit allowed in New Zealand freshwaters. Future risk assessments and toxicity tests need to consider indirect impacts resulting from infections to invertebrate and vertebrate species penetrated by cercariae and serving as second intermediate hosts of trematodes.

1. Introduction

There is now solid evidence that anthropogenic pollutants can directly or indirectly affect levels of parasitic infections in aquatic organisms (Poulin, 1992; Lafferty, 1997; Blanar et al., 2009). Indeed, the sensitivity of many parasites makes them reliable indicators of environmental impact from various pollutants (Sures, 2004; Vidal-Martinez et al., 2010). The growing interest in the ways pollution and parasitism may interact has led to the identification of various processes through which pollutants can boost infection levels by specific parasites (Morley, 2010). For example, host immunity can be weakened by pollution stress, rendering exposed animals more susceptible to infection (Morley et al., 2006; Rohr et al., 2008a).

In some parasites, the multiplication rate of the parasite and the production of infective propagules may also be influenced by pollution. Trematodes are particularly likely to be affected in this way. Common in most aquatic habitats, trematodes are parasitic flatworms with complex life cycles (Kearn, 1998). Adult worms live inside a vertebrate definitive host, such as fish, amphibians or aquatic birds. Their eggs are released in water through host faeces, where they hatch into larvae that seek and infect a snail, which acts as first intermediate host. Within the snail, the parasite multiplies asexually to produce and release numerous free-swimming infective stages known as cercariae. Short-lived and non-feeding, these cercariae proceed to infect the parasite's second intermediate host, which is either an invertebrate or a small vertebrate, depending on the trematode species; cercariae encyst within this second intermediate host to await predation by the definitive host. In trematode life cycles, snails act as sources of infective stages which then go on to penetrate and exploit other organisms. Both the quantity and quality of cercariae emerging from snails determine the risk of infection for these other organisms. The rates at which cercariae are produced within, and released from, snails are known to be extremely sensitive to abiotic conditions, such as temperature or salinity (Mouritsen, 2002; Poulin, 2006; Thieltges and Rick, 2006; Studer et al., 2010; Lei and Poulin, 2011). Similarly, cercarial survival and infectivity are also affected by external conditions (Pietrock and Marcogliese, 2003). The sensitivity of cercarial out-

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put and survival to abiotic factors suggests another potential impact of pollution on parasitism: the presence of pollutants in water may affect the production of cercariae, as well as influence how long they survive to find and infect their target host.

Some forms of pollution can enhance snail population densities, or cause direct immunosuppression of the next host in the trematode life cycle, thereby promoting higher infection levels or greater pathogenicity (Kiesecker, 2002; Johnson et al., 2007; Rohr et al., 2008b). In addition, a growing number of studies (e.g., Cross et al., 2001; Morley et al., 2001, 2002; Koprivnikar et al., 2006; Koprivnikar and Walker, 2011) have examined the effect of pollutants on either the output of cercariae from snails or their subsequent survival. For instance, Kelly et al. (2010a) have recently shown that New Zealand snails *Potamopyrgus antipodarum* infected by the trematode *Telogaster opisthorchis* and exposed to moderate concentrations of the common herbicide glyphosate, release approximately three times more cercariae per day than snails kept in glyphosate-free water. Although it has limited impact on adult fish (Poulin, 1993), *T. opisthorchis* causes spinal malformations and increased mortality in juveniles of the fish species, some of which are threatened, serving as its next hosts (Kelly et al., 2010b). The interaction between trematode parasitism and glyphosate pollution from agricultural run-off may thus have substantial consequences.

Glyphosate (*N*-phosphonomethyl-glycine) is a broad-spectrum inhibitor of amino acid synthesis in plants, and the most widely-used herbicide in the world (Relyea, 2005a; Kolpin et al., 2006). Glyphosate adsorbs to soil particles but part of what is applied to vegetation is nevertheless dispersed from soil to freshwaters by wind, surface run-off or soil leachate (Pérez et al., 2007; Siemering et al., 2008). Glyphosate is often applied as the commercial formulation Roundup®, where it is combined with the surfactant polyoxyethyleneamine (POEA) whose function is to increase penetration through plant cuticle. While glyphosate is only toxic to aquatic vertebrates at high concentrations, POEA is often the primary toxic agent (Folmar et al., 1979; Tsui and Chu, 2003; Relyea, 2005b). The potential for glyphosate to impact host-parasite interactions in freshwater ecosystems therefore goes beyond the findings of Kelly et al. (2010a) based on a single trematode species.

In New Zealand, the snail *P. antipodarum* serves as hosts to at least a dozen trematode species in addition to *T. opisthorchis* (Winterbourn, 1973). It is therefore possible that glyphosate pollution impacts the proliferation of several parasite species, with consequences for numerous invertebrate and vertebrate populations serving as subsequent hosts for these parasites. In this study, we consider three of these additional trematode species. The first, *Coitocaecum parvum* (Opecoelidae), infects amphipods as second intermediate hosts and eleotrid fishes as definitive hosts, whereas the second species, *Apatemon* sp. (Strigeidae), uses eleotrid fishes as second intermediate hosts and ducks as definitive hosts. Both of these typically reach high abundances in those hosts (Lagrue and Poulin, 2008; Herrmann and Poulin, 2011), and are therefore important parasites in New Zealand freshwater ecosystems. The third study species is an undescribed species of the family Renicolidae. Although relatively common in snails, its life cycle remains unknown; however, if it follows the typical renicolid pattern, the second intermediate host should be a fish and the definitive host should be a piscivorous bird.

The aim of this study is to test for indirect effects of the herbicide glyphosate and its surfactant POEA on trematode parasites using the snail *P. antipodarum* as intermediate host in freshwater habitats of New Zealand's South Island. More specifically, our objectives are (i) to quantify the impact of snail exposure to different concentrations of glyphosate on cercarial production and output, for three different trematode species; and (ii) to assess the effect of exposure to different glyphosate concentrations on cercarial survival in one of those trematode species.

2. Methods

2.1. Snail collection and laboratory processing

To obtain snails infected with different trematode species, we collected snails from three different locations: (i) the upper portion of Tomahawk Lagoon (45°54′S, 170°32′E), a slightly brackish lake within the Dunedin city limits, (ii) Lake Waihola (46°01′S, 170°05′E), a coastal lake receiving brackish water on regular tidal cycles from the nearby Pacific Ocean, and (iii) a section of the Koau Branch of the Clutha River (46°17′S, 169°45′E), about 10 km from where it drains into the Pacific Ocean. Snails were obtained in the austral summer between December 2010 and February 2011, by dragging a dipnet through macrophytes and sediment within a few metres from the shore. Water and fresh macrophyte (*Ruppia polycarpa*) were also taken from the site of collection for maintenance of snails in the laboratory.

Only snails with shell lengths between 4 and 5 mm were retained, to limit size variability but also because this adult size-class has the highest prevalence of infection. To identify snails infected with trematodes, snails were placed individually into 12-well flat bottom culture plates (volume 3 mL) filled with 2 mL of freshwater and incubated for two hours at 25 °C, stimulating cercariae to emerge. Each snail was then screened for infection under a dissecting microscope. This was repeated over four consecutive days. Once parasitised snails were identified, uninfected snails and those harbouring the different focal trematode species were separated and placed in 2L tanks with aerated freshwater and pieces of the macrophyte *R. polycarpa* as food, and kept at 12 °C until they were used in the experiment, with water and fresh macrophytes replaced regularly.

The infected snails used in this study consisted of snails from the Clutha River infected with *C. parvum*, snails from Tomahawk Lagoon infected with *Apatemon* sp., snails from Tomahawk Lagoon infected with the renicolid, and snails from Lake Waihola infected with the renicolid. The renicolids from Tomahawk Lagoon and Lake Waihola were confirmed to belong to the same species based on genetically similar 28S ribosomal DNA sequences (I. Blasco-Costa, unpublished data).

2.2. Glyphosate treatments

Four water treatments were used in the present study, consisting of a glyphosate-free control and three different concentrations of the glyphosate and POEA mixture. Because glyphosate and the surfactant POEA come in a mixed concentration of 360 mg L^{-1}, dilution was required to get the desired concentrations. The three treatments were made using aged and filtered freshwater (from site of collection) and the commercial formulation Glyphosate 360 (360 mg L^{-1} plus 10–20% POEA; supplied by Ravensdown, New Zealand). Each of the three distinct glyphosate treatments was diluted to either: (1) low concentration, 0.36 mg a.i. L^{-1}, (2) medium concentration, 3.6 mg a.i. L^{-1}, or (3) high concentration, 36 mg a.i. L^{-1}. These concentrations fall within the range recorded in natural freshwaters (e.g. Giesy et al., 2000; Pérez et al., 2007; Battaglin et al., 2009), and were chosen to match those used in the previous study on the effect of glyphosate on trematodes of the snail *P. antipodarum* (Kelly et al., 2010a). For comparison, the environmental exposure limit for New Zealand freshwaters has been set at 0.37 mg a.i. L^{-1} by New Zealand's Environmental Risk Management Authority (ERMA NZ, 2005), the manufacturer's recommended maximum concentration near water in North America is 3.8 mg a.i. L^{-1} (see Relyea, 2005b), and our highest concentration matches levels considered toxic to amphibians and fish (Folmar et al., 1979; Relyea, 2005a).

2.3. Cercarial output experiments

All four groups of snails (*C. parvum*-infected snails, *Apatemon*-infected snails, renicolid-infected snails from Tomahawk, and renicolid-infected snails from Waihola) were used in these experiments, though in separate trials run at slightly different times.

Snails were placed individually into wells of a 24-well plate (single well volume = 3.3 mL), and randomly allocated to each of the four treatments. Low prevalence of infection in natural snail populations limited the sample size. Because of a shortage of infected snails, the high glyphosate concentration (36 mg a.i. L^{-1}) treatment was only used for renicolid-infected snails. In total, we used 18 *C. parvum*-infected snails (6 each in the control, low glyphosate and medium glyphosate treatments), 9 *Apatemon*-infected snails (3 each in the control, low glyphosate and medium glyphosate treatments), 48 renicolid-infected snails from Tomahawk (12 each in the control, low glyphosate, medium glyphosate and high glyphosate treatments), and 36 renicolid-infected snails from Waihola (9 each in the control, low glyphosate, medium glyphosate and high glyphosate treatments).

Each well was filled with 2.1 mL of either control water or one of the glyphosate solutions, and the plates were maintained at approx. 20 °C under a natural photoperiod. Cercariae emerged from each snail were counted daily for 28 consecutive days, at the same time every morning, using a dissecting microscope. After the daily count, snails were moved to a new plate with each well filled with fresh water (control or glyphosate solutions, depending on the snail), and small pieces of macrophytes from the same collection site were added as food once per week. After the completion of the experiments, snails were dissected to confirm their infection status and identify any concurrent infection by other trematode species; in total, only two snails had to be excluded for the latter reason.

2.4. Cercarial survival experiment

For this experiment, only renicolid-infected snails from Tomahawk were used. Again, snails were maintained individually in wells of a 24-well plate (single well volume = 3.3 mL), and randomly allocated to each of the three water treatments (the high glyphosate concentration, 36 mg a.i. L^{-1}, was not used in this experiment). In total, we used 24 renicolid-infected snails from Tomahawk (8 each in the control, low glyphosate, and medium glyphosate treatments). Each well was filled with 2.1 mL of either control water or one of the glyphosate solutions, and the plates were maintained at approx. 20 °C under a natural photoperiod. On the day of the experiment, the snails were examined regularly under a dissecting microscope, and were removed from the well as soon as a few cercariae emerged from them, to ensure that cercarial survival could be measured on freshly-emerged individuals. Each snail released between 1 and 5 cercariae, for a total of 61 cercariae, consisting of 21 in the control treatment, 19 in the low glyphosate treatment, and 21 in the medium glyphosate treatment. From the time the snail was removed from its well (time zero), cercariae were monitored every hour individually under a dissecting microscope to measure the duration of their survival, with death defined as complete cessation of movement.

2.5. Data analysis

Each analysis was tailored to the data, and chosen to provide the most appropriate distribution of residuals. All tests were performed using the statistical program JMP 9.0.2, with results considered significant at $P < 0.05$.

For the cercarial output experiments, the factors included in the models were treatment (control, low glyphosate, medium glyphosate, high glyphosate), snail identity nested within treatment, and day of observation. As mentioned above, trials using *C. parvum*- and *Apatemon*-infected snails did not include exposure to the high glyphosate concentration. For each model below, the error distribution that best fitted the data was determined based on AIC (Akaike information criterion) values. The cercarial output data ($\log x + 1$ transformed) from *C. parvum*-infected snails were analysed with a generalised linear model (GLM), with a normal error structure and an identity link function. The cercarial output data ($\log x + 1$ transformed) from *Apatemon*-infected snails and from renicolid-infected snails from Waihola were analysed using GLMs, with a Poisson error structure and a log link function. In the above analyses, the numbers of cercariae released per snail per day were the response variable. However, for renicolid-infected snails from Tomahawk, because the data had a large number of zeros (i.e. no cercarial shedding by a particular snail on a given day), the cercarial output data were analysed with a logistic regression model. The numbers of cercariae released per snail per day were used as response variable, and treated as an ordinal variable with values of 0 (no cercariae), 1 (1 cercaria), 2 (2 cercariae), 3 (3 cercariae), 4 (4–5 cercariae), 5 (6–9 cercariae) or 6 ($\geqslant$10 cercariae). In all above analyses, post-hoc Tukey–Kramer tests were used for pair-wise comparisons to determine which treatment means were different from one another.

For the cercarial survival experiment, because of the marked bimodal distribution of cercarial lifespan, the survival data were analysed with a logistic regression model. The lifespan of cercariae was used as a response variable, and treated as an ordinal variable with values of 1 (35 h or less), or 2 ($\geqslant$40 h). The factors included in the model were treatment (control, low and medium glyphosate concentrations), and snail identity nested within treatment.

3. Results

3.1. Cercarial output

The numbers of cercariae released by snails varied between trematode species, ranging from roughly 1–4 per day for the renicolid, to 5–20 per day for *C. parvum* and *Apatemon* sp. Our analyses revealed very similar patterns of cercarial output for all trematode species considered here (Table 1). There were significant differences in cercarial output among individual snails, and in most cases the daily numbers of cercariae exiting a snail changed over

Table 1
Results of statistical models (see Methods) testing the effects of treatment (exposure to control water versus different glyphosate concentrations), snail identity nested within treatment, and the day of observation, on the number of cercariae emerging from infected *P. antipodarum* snails. Results are shown for the trematode species *C. parvum*, *Apatemon* sp. and two populations of an undescribed renicolid.

Trematode species	Factor	Degrees of freedom	Chi-squared	*P*
C. parvum	Treatment	2	99.14	<0.0001
	Snail [treatment]	15	131.31	<0.0001
	Day	1	23.95	<0.0001
Apatemon sp.	Treatment	2	16.79	0.0002
	Snail [Treatment]	6	23.38	0.0007
	Day	1	0.58	0.4455
Renicolid (Tomahawk)	Treatment	3	13.18	0.0043
	Snail [Treatment]	30	51.24	0.0092
	Day	1	36.60	<0.0001
Renicolid (Waihola)	Treatment	3	6.94	0.0738
	Snail [Treatment]	41	162.93	0.0001
	Day	1	47.41	<0.0001

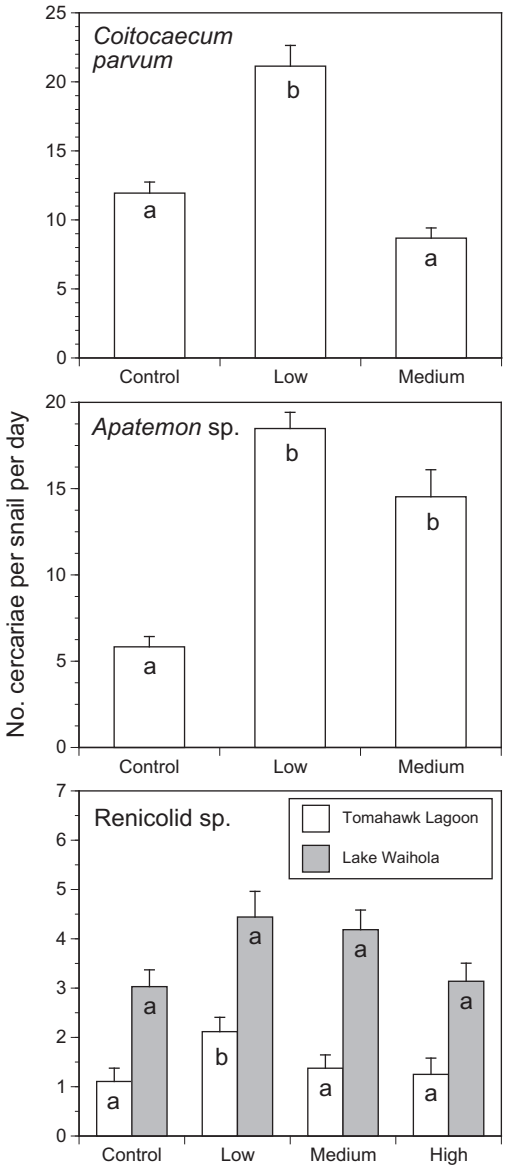

Figure 1. Mean (±SE) number of cercariae emerging per day from individual snails, *P. antipodarum*, exposed to either control water, low, medium or high concentrations of the herbicide glyphosate. Data are shown separately for the trematodes *C. parvum* ($N = 6$ snails for each treatment), *Apatemon* sp. ($N = 3$), and an undescribed renicolid species from two localities (Tomahawk Lagoon, $N = 12$; Lake Waihola, $N = 9$). In each case, different letters on the bars indicate mean values that are significantly different (Tukey–Kramer tests, $P < 0.05$).

the duration of the experiment. Except in the case of *Apatemon*-infected snails, the average daily cercarial output dropped by about 10–50% over the 28 experimental days. However, for all trematode species, there was an effect of treatment, i.e. the presence and concentration of glyphosate, on cercarial output (Table 1). This effect was only marginal in the experiment using renicolid-infected snails from Waihola, but strongly significant in all other experiments. In all cases, the daily cercarial output of snails exposed to low (or medium) concentrations of glyphosate was higher than that of snails in control water or exposed to higher glyphosate concentrations (Fig. 1). Compared to the numbers of cercariae released per day by snails under control conditions, those released by snails exposed to low glyphosate concentrations were about 1.5–3 times higher, depending on the species.

Table 2
Results of a logistic regression testing the effects of treatment (exposure to control water, low or medium glyphosate concentrations) and snail identity nested within treatment, on the survival time (in hours) of renicolid cercariae emerging from infected *P. antipodarum* snails.

Factor	Degrees of freedom	Chi-squared	*P*
Treatment	2	42.26	<0.0001
Snail [treatment]	21	16.91	0.7163

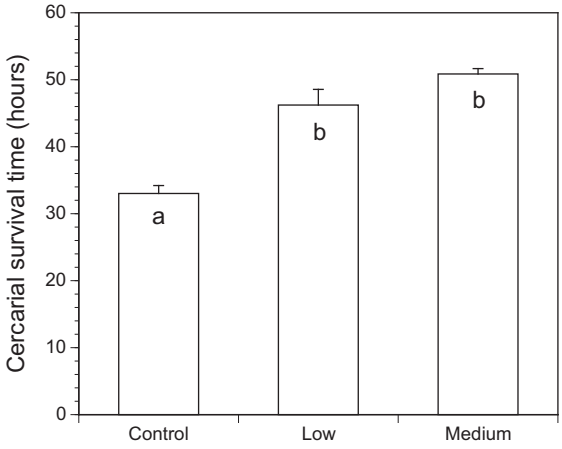

Figure 2. Mean (±SE) survival time of renicolid cercariae emerging from the snail, *P. antipodarum*, exposed to either control water ($N = 21$ cercariae), low ($N = 19$), or medium ($N = 21$) concentrations of the herbicide glyphosate. Different letters on the bars indicate mean values that are significantly different (Tukey–Kramer tests, $P < 0.05$).

3.2. Cercarial survival

Renicolid cercariae survived from 27 to 60 h under experimental conditions. The identity of the snail from which they emerged had no influence on the survival time of cercariae (Table 2). In contrast, there was a statistically significant effect of the treatment, i.e. the presence and concentration of glyphosate, on cercarial survival (Table 2). Survival times were shortest under control conditions and longest under exposure to medium glyphosate concentrations (Fig. 2). On average, survival was extended by almost 50%, i.e. about 17 h, in the medium glyphosate concentration compared to control water.

4. Discussion

Anthropogenic stress from agricultural run-off has major impacts on freshwater ecosystems (Relyea, 2005b; Danz et al., 2007). Although still somewhat neglected, pollution-mediated increases in parasitism may represent one of the most important indirect effects of herbicides entering freshwaters. Here, we have shown that exposure of infected *P. antipodarum* snails to low to medium concentrations of the widely-used herbicide glyphosate led to the production and release of increased numbers of cercariae by three different trematode species, and that the survival of cercariae from one of those trematodes is also enhanced under exposure to moderate glyphosate concentrations. Taken together, and assuming cercarial infectivity is not impaired, these results suggest that glyphosate contamination of New Zealand freshwaters could promote higher infection levels in the invertebrate and vertebrate species penetrated by cercariae and serving as second intermediate hosts of trematodes.

Although the few snails exposed to the high glyphosate concentration (36 mg a.i. L^{-1}) in this study survived, medium-to-long-term exposure to this concentration is generally lethal to *P. antipodarum* (Kelly et al., 2010a). In contrast, snails exposed to the low and medium concentrations, i.e. 0.36 and 3.6 mg a.i. L^{-1}, survived well the month-long exposure in our study and in a previous study (Kelly et al., 2010a). Similar concentrations have been recorded in natural freshwaters (e.g. Giesy et al., 2000; Pérez et al., 2007; Battaglin et al., 2009), and range from the environmental exposure limit for New Zealand freshwaters set by New Zealand's Environmental Risk Management Authority (ERMA NZ, 2005) to the manufacturer's recommended maximum application rate (Relyea, 2005b). These concentrations are generally below levels considered toxic for most aquatic organisms (Folmar et al., 1979; Relyea, 2005a; Pérez et al., 2007; Siemering et al., 2008), and yet it is at these concentrations that cercarial output from trematode-infected snails peaked in our study. Our results support those of earlier studies (Morley et al., 2003; Johnson et al., 2007; Rohr et al., 2008b) by providing a clear demonstration that the potentially negative impact of pollutants should consider interspecific interactions and not just direct effects in oversimplified single-species exposure experiments.

In most cases, exposure to pollutants such as metals, pesticides and herbicides reduces both multiplication of trematodes within snails (e.g., Hira and Webbe, 1972; Yescott and Hansen, 1976) and/or their rate of emergence from snails (e.g., Morley et al., 2003; Koprivnikar and Walker, 2011). In contrast, we found that month-long exposure to environmentally realistic concentrations of glyphosate results in up to three-fold increases in the number of cercariae emerging from snails. A possible explanation is that exposure to glyphosate weakens the snail in ways that facilitate the acquisition of host resources by the trematode and their conversion into cercariae. For instance, the direct negative impacts of the pollutant on snail physiology may impair its ability to resist parasite exploitation. The most remarkable feature of our results is that the same pattern is seen in all three trematode species tested. This echoes the findings of Rohr et al. (2008b), who also observed a similar consistency of responses across different trematode species to the herbicide atrazine. If we add to these *T. opisthorchis*, the species tested earlier by Kelly et al. (2010a), we now have four trematodes from different families (Opecoelidae, Strigeidae, Renicolidae, and Cryptogonimidae) showing enhanced cercarial output when their common snail host is exposed to low to medium concentrations of glyphosate. Since the pattern is not species-specific, a direct effect of glyphosate on the snail with indirect benefits to the parasites seems the most likely mechanism. We might therefore expect similar increases in cercarial output in all the other trematode species (see Winterbourn, 1973) using the snail *P. antipodarum* as intermediate host. Furthermore, under natural conditions periphyton production is enhanced under exposure to medium glyphosate concentrations because of greater availability of phosphorus (Pérez et al., 2007); this should result in greater food resources for snails, and in turn more resources to be converted into cercariae by trematodes (Johnson et al., 2007).

As for cercarial output, direct exposure to a range of pollutants may also, though not necessarily, affect cercarial survival (e.g., Cross et al., 2001; Morley et al., 2001; Koprivnikar et al., 2006). However, here we found that exposure to environmentally realistic concentrations of glyphosate resulted in longer survival by renicolid cercariae. The weakening of the snail host postulated to explain enhanced cercarial output in the presence of glyphosate could possibly also lead to the production of qualitatively superior cercariae. Cercariae do not feed, and their lifespan is determined by their glycogen reserves (Lawson and Wilson, 1980). However, our results cannot be used to determine whether cercariae emerging from glyphosate-exposed snails are also better-provisioned with glycogen stores, in part because snails were only maintained in control water or glyphosate solutions long enough to produce a few cercariae. Alternatively, our results may indicate a direct effect of glyphosate on cercarial survival, for which we cannot provide a plausible mechanism. Although we did not continuously monitor all cercariae, there were no visible differences in swimming or activity of cercariae among different treatments during the regular observation periods, suggesting that the longer lifespan under glyphosate exposure is not merely the result of reduced activity. The longer lifespan of glyphosate-exposed cercariae might thus reflect a longer window of infectivity, although additional tests will be necessary to confirm that second intermediate hosts would indeed face a greater infection risk (see McCarthy, 1999; Morley et al., 2002, 2003; Koprivnikar et al., 2006). In contrast to our findings, Rohr et al. (2008a) found no effect of glyphosate on the survival of *Echinostoma trivolvis* cercariae. However, their study used exposure to glyphosate only, without the surfactant POEA, suggesting that perhaps POEA itself and not the herbicide is the cause of the effects on cercarial output and survival observed here.

In conclusion, our study demonstrated (i) significant increases in the output of infective stages of three different trematode species from snails exposed to low or medium concentrations of the herbicide glyphosate, and (ii) significant increases in the survival of cercariae from one of those species under glyphosate exposure. We acknowledge that because of the heterogeneous nature of freshwater systems, glyphosate concentrations may only reach the levels at which trematodes are affected in some limited areas. Nevertheless, glyphosate, as the active ingredient in the commercial formulation Roundup®, is widely used in agriculture, forestry, nurseries and home gardens for the control of herbaceous plants, making it the most widely-used herbicide in the world (Relyea, 2005a; Kolpin et al., 2006). What is of concern is that the effects we measured involve glyphosate concentrations within the environmental exposure limit set by New Zealand's Environmental Risk Management Authority (ERMA NZ, 2005). Such allowable limits are established based on standard toxicity tests that overlook interactions with parasitism or other naturally occurring stressors, and therefore underestimate ecological risks. Predicting the environmental impact of herbicides or other pollutants cannot be achieved from single-species toxicity tests (Pratt et al., 1997). We echo other researchers (Kiesecker, 2002; Rohr et al., 2008a,b; Kelly et al., 2010a; Morley, 2010) in strongly recommending that future risk assessments include an evaluation of pollutant impact on all aspects of host-parasite interactions, from the production, survival and infectivity of parasites to the susceptibility of hosts and the fitness reductions they may incur.

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