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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).