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Contaminant mixtures interact to impair predator-avoidance behaviours and survival in a larval amphibian

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ABSTRACT

Global declines in amphibian populations are a significant conservation concern, and environmental contamination is likely a contributing driver. Although direct toxicity may be partly responsible, contaminants are often present at sub-lethal concentrations in the wild. Behavioural end-points are becoming an increasingly useful method to estimate the impact of contaminants, particularly if the behavioural responses manifest to affect individual fitness (i.e. survival, growth, or reproduction). In the wild, most animals are affected by multiple stressors, and determining how these interact to affect behaviour is critical for understanding the ecological implications of contaminant exposure. Here, we examined the individual and interactive effect of the heavy metal copper and the insecticide imidacloprid on mortality rates and anti-predator behaviours of spotted marsh frog (*Limnodynastes tasmaniensis*) tadpoles. This common species frequently occupies and breeds in contaminated stormwater and agricultural wetlands, where copper and imidacloprid are often present. These contaminants may alter behaviour via physiological and neurological pathways, as well as affecting how tadpoles respond to chemical cues. Tadpoles suffered unexpectedly high mortality rates when exposed to imidacloprid concentrations well below published LC50 concentrations. Only unexposed tadpoles significantly avoided predator cues. Copper and imidacloprid reduced swimming speed and distance, and escape responses, while increasing erratic swimming. We observed an interactive effect of imidacloprid and copper on erratic swimming, but in general imidacloprid and copper did not act synergistically. Our results suggest that as contaminants enter waterbodies, tadpoles will suffer considerable direct mortality, reduced foraging capacity, and increased susceptibility to predation. Our results provide the first evidence of imidacloprid affecting amphibian behaviour, and highlight both the adverse effects of copper and imidacloprid, and the importance of exploring the effect of multiple contaminants simultaneously.

1. Introduction

Humans have caused unprecedented rates and scales of environmental change, with considerable impacts on wildlife (Pereira et al., 2010; Vitousek et al., 1997). These impacts are often assessed via changes in abundance or species richness (Sievers et al., 2018a). Although these population- and community-level metrics indicate that animals are present, they offer little information regarding functional responses to environmental change (Palmer and Febria, 2012), and may provide misleading information on the value of habitats since these metrics do not incorporate survival and reproductive measures (Sievers et al., 2018a).

When the environment changes, animals often respond initially by altering their behaviour, and this can be pivotal in determining how successful animals are in these new conditions (Tuomainen and Candolin, 2011; Wong and Candolin, 2015). One reason this is important is that behavioural responses are closely linked to fitness (i.e. survival, growth and reproduction), particularly when considering contaminant-induced behavioural change (Montiglio and Royauté, 2014; Zala and Penn, 2004). The sensitivity of many behaviours may also allow sub-lethal effects to be detected at lower levels of contamination than those typically needed to observe direct reductions in fitness (Zala and Penn, 2004). Consequently, behavioural end-points are becoming increasingly useful for estimating the impact of contaminants within natural systems.

Amphibians are one of the most imperilled taxa (Monastersky, 2014), and often live and breed in locations that are designed to receive contaminated water (e.g. stormwater wetlands receiving urban runoff; Brand and Snodgrass, 2010) or located in areas inherently prone to intentional contamination (e.g. agricultural wetlands receiving

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pesticides and fertilisers; Hazell et al., 2001). Amphibians in general, and their aquatic larval stages in particular, are thus often exposed to a variety of contaminants that affect fitness (Egea-Serrano et al., 2012) and behaviour (Shuman-Goodier and Propper, 2016). For example, heavy metals can affect tadpole swimming by reducing sprint speeds and distances, and causing erratic swimming, behavioural alterations which may affect fitness by increasing predation risk (Brunelli et al., 2009; Hayden et al., 2015).

Amphibians use chemical cues to locate resources, detect conspecifics and avoid predators (Lürling and Scheffer, 2007). Exposure to contaminants can affect responses to cues in other taxa by compromising sensory acuity (e.g. copper can damage the olfactory bulb; Tierney et al., 2010) or by changing the characteristics of the cues (e.g. humic acid can bind to conspecific cues; Fisher et al., 2006). Contaminant exposure may also cause amphibians to fail to perceive or respond to important cues. For example, tadpoles exposed to a sublethal concentration of the herbicide glyphosate exhibited impaired responses to olfactory cues from injured conspecifics – an indicator of predation risk – due to the loss of function of alarm cues (Moore et al., 2015). Coined 'info disruptors' (Lürling and Scheffer, 2007), these contaminants – which typically act at concentrations well below those examined in standard ecotoxicological tests – may represent a significant threat to amphibians. Therefore, contaminants can impact growth, development and survival directly through toxicity and the resulting morphological or physiological changes (Egea-Serrano et al., 2012), or indirectly by impairing behaviours which can increase vulnerability to predators and reduce foraging efficiency (Bridges, 1999).

Most animals are affected by multiple stressors (Jackson et al., 2016; Ormerod et al., 2010), such as different classes of chemical contaminants (e.g. heavy metals and pesticides). Determining how these interact to impact behaviour is critical for understanding the ecological implications of contaminant exposure under natural conditions (Halfwerk and Slabbekoorn, 2015), yet it is more common to study the effect of individual, rather than combined, stressors. Multiple stressors can act: synergistically (outcome is stronger than predicted based on the sum of the individual effects); antagonistically (outcome is weaker than predicted based on the sum of the individual effects); additively (equal to the sum of the individual effects, i.e. no interaction); or even produce an 'ecological surprise', such as when two stressors exhibit no effects in isolation, but do together (Crain et al., 2008; Hale et al., 2017). Given that the various interaction types could have very different ecological consequences, we need to investigate responses to combinations of stressors that occur in nature.

Here, we examined the individual and interactive effects of copper and the insecticide imidacloprid on anti-predator behaviours of spotted marsh frog (*Limnodynastes tasmaniensis*) tadpoles. This common species frequently occupies and breeds in stormwater and agricultural wetlands throughout much of Australia (Hamer and Parris, 2011; Hazell et al., 2001). We previously showed that it responds to olfactory cues from predatory dragonfly larvae, and that this response can be affected by chronic exposure to contaminated natal environments (Sievers et al., 2018b). Copper and imidacloprid often contaminate wetlands in Australia (Allinson et al., 2015, 2017) and around the world (Unrine et al., 2007; Weston et al., 2009). Imidacloprid acts on the central nervous system (Gervais et al., 2010), and other neurotoxic insecticides are known to affect normal swimming behaviours of amphibians (e.g. Brunelli et al., 2009). To our knowledge, no previous study has focused on behavioural changes caused by exposure to imidacloprid or the interaction between this insecticide and copper.

We conducted short-term laboratory exposures at environmentally relevant concentrations followed by choice experiments to document behavioural responses to predator olfactory cues and tail prodding (i.e. a simulated 'attack'; Pauli et al., 1999; Van Buskirk and McCollum, 2000). We predicted that tadpoles exposed to contaminants, especially copper and imidacloprid, would be less able to recognise and avoid predators than unexposed tadpoles. We also predicted that pollutant exposure would compromise the swimming behaviour of tadpoles, which could enhance predation risk as sprint speed and distance can be correlated with the ability of a tadpole to escape predators (Bridges, 1999).

2. Materials and methods

2.1. Study species

The spotted marsh frog *Limnodynastes tasmaniensis* (Günther, 1858) is a ground-dwelling frog native to Australia. We collected a portion of nine egg masses from an isolated wetland (37°37′08.06″ S, 145°00′10.67″ E) created for the endangered growling grass frog *Litoria raniformis* (Keferstein, 1867). We hatched eggs and reared tadpoles in RO/DI water with artificial salt medium within individual 2 L, acid-washed containers, and fed tadpoles crushed flake food and lettuce until experimentation at 23 days old (mean length ± SE: 9.02 ± 0.21 mm, n = 20).

2.2. Creation of predator cues

We created predator cues using locally collected dragonfly larvae (Suborder: Epiprocta); a ubiquitous and voracious tadpole predator commonly used in predator detection and avoidance experiments (Hanlon and Relyea, 2013; Sievers et al., 2018b). To harvest predator odours, we kept 36 dragonfly larvae in 4 L of aged tap water for 18 h, which was subsequently diluted to 20 L to achieve a final concentration of 1 larva per 0.56 L of odour water (similar to Carlson and Langkilde, 2013; Ehrsam et al., 2016). All cue water was made fresh daily and used within 12 h.

2.3. Contaminant preparation and exposure

Much of the copper within urban stormwater wetlands comes from vehicles, especially brake pads and tyres (Sansalone and Buchberger, 1997). The neonicotinoid insecticide imidacloprid is primarily registered in Australia for use against general household insects, insect pests of fruits and vegetables, and termites (Allinson et al., 2015), and has a high potential to enter urban wetlands due to its high solubility and persistence in water. Although widely studied for its unintended impact on bees (Cresswell, 2011), very few studies have investigated its impact on amphibians.

We conducted exposures using a fully crossed, 3 × 3 experimental design. We dosed copper at 0, 10 and 20 μg/L, matching levels found within urban wetlands (Allinson et al., 2017) and below lethal concentrations for other amphibians (Barry, 2011; Chen et al., 2007). We dosed imidacloprid at 0, 0.25 and 0.50 μg/L, again matching levels occurring within urban wetlands (Allinson et al., 2015; Weston et al., 2009), and below lethal concentrations for other amphibians (Feng et al., 2004; Quan et al., 2006).

We made up stock solutions of copper (from $CuNO_3$ salt; nominal 5 mg/L) and imidacloprid (as the commercial formulation Confidor 200SC (Bayer), 50 g/kg active ingredient; nominal 10 μg/L). During the trial period, we diluted stock solutions daily with RO/DI water, and samples from each treatment were analysed by SymBio Laboratories (Eight Miles Plains, QLD, Australia).

We exposed tadpoles for approximately 24 h within 2 L acid-washed containers. This time period was chosen following Hayden et al. (2015), who observed behavioural modifications following 24-h exposures to copper in wood frogs (*Lithobates* (*Rana*) *sylvatica*). Morning and afternoon batches of tadpoles received their treatments 6 h apart to keep exposure time consistent.

2.4. Experimental trials

We conducted trials within plastic choice tanks (38 × 17 × 13 cm;