

Research Article

Ontogenetic shift in toxicity of invasive cane toads facilitates learned avoidance by native predators

Matthew J. Greenlees* and Richard Shine

School of Life and Environmental Sciences A08, University of Sydney, NSW 2006, Australia

Present address: Department of Biological Sciences Macquarie University, NSW 2109, Australia

E-mail: matthew.greenlees@sydney.edu.au (MJG), rick.shine@mq.edu.au (RS)

*Corresponding author

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OPEN ACCESS**Abstract**

The ecological impact of an invasive species depends upon many factors. Our experimental studies show that exposure to the tadpoles of cane toads (containing less toxin), *Rhinella marina*, induces predatory native frogs, *Litoria dahlii*, to avoid metamorph cane toads (containing comparatively more toxin) in subsequent encounters. The frog's ability to generalise its aversion response from tadpoles to metamorphs, coupled with the toad's strong ontogenetic variation in toxin content, which reflects its multiphasic life history, thus play critical roles in enabling native predatory frogs to survive the cane toad invasion.

Key words: stimulus generalisation, learning, invasive species, *Bufo marinus*, anuran

Introduction

Despite widespread concern about the ecological impacts of invasive species, factors that influence vulnerability of native species to invaders remain poorly understood (Phillips et al. 2003; Schüttler et al. 2009; Brown et al. 2013). One clear-cut type of impact involves the spread of poisonous plants or animals that kill native herbivores or predators that attempt to consume the newcomers (Brodie and Brodie 1999; Crossland et al. 2008). In such a system, the magnitude of ecological impact may vary widely among native taxa, as a function of physiological resistance to the toxin and behavioural responses to the novel food type (Smith and Phillips 2006; Greenlees and Shine 2011; Chen et al. 2016). Both the nature and magnitude of impact, as well as the mechanism via which predators respond to the novel species, can have profound effects on the local ecosystem (Carlsson et al. 2009). If some individuals of the invasive taxa are poisonous enough to induce illness but not death in the animals that consume them, individuals may be selected for their capacity to incorporate the novel prey into their diet (e.g. Robbins et al. 2013), or aversion learning may enable those individual predators to avoid the invader in subsequent encounters and hence survive (Webb et al. 2008; Ward-Fear et al. 2016).

What attributes of the invader and the native predator influence the effectiveness of such aversion learning? Plausibly, the most critical issue in the case of the invader involves variation among individuals in toxin content. If every individual contains too little toxin to be fatal, then aversion is irrelevant to predator survival at least in the short term (although it may have longer-term consequences if the toxin has sublethal effects: e.g. Llewelyn et al. 2009). If every individual of the invasive species is fatally toxic, there are no opportunities for learned aversion. In contrast, an invasive species that includes individuals spanning a wide range of toxin levels is more likely to induce aversion, when low-toxicity individuals are consumed, that enables the predator to avoid a subsequent ingestion event that would otherwise be fatal. Predator attributes will also influence outcomes (Chen et al. 2016). Two important aspects are the predator's ability to learn rapidly, and to develop a generalised aversion to the toxic invader in response to an initial encounter. Predators that require many "training" episodes to learn aversion, or who avoid only items that closely resemble the already-encountered toxic "prey", are less likely to survive.

The spread of toxic cane toads (*Rhinella marina* Linnaeus, 1758) through tropical Australia provides an opportunity to test these ideas. Many native predators (including snakes, lizards, crocodiles, marsupials) are fatally poisoned when toads invade (Burnett 1997; Griffiths and McKay 2007; Letnic et al. 2008). Although native frogs also attack toads, and sometimes die in the process (Shine et al. 2009), most frog populations are unaffected (Freeland and Kerin 1988; Catling et al. 1999) because frogs rapidly learn to avoid the toxic toads (Shine et al. 2009; Greenlees et al. 2010). We speculated that vulnerable predators might learn taste-aversion to toxic toads if they encountered relatively non-toxic early life-stages of toads prior to encountering more toxic later stages. To test this prediction, we conducted experimental trials. Throughout the terrestrial phase of their lives, Dahl's Aquatic frogs (*Litoria dahlia*) prey on both the aquatic and terrestrial stages of the toad; toxin levels are lower in the former stage than the latter (Crossland et al. 2008; Shine et al. 2009). We predicted that a predator's experience with toad larvae would affect the outcomes of its subsequent encounters with the more toxic terrestrial-phase toads.

Materials and methods

In the field, juvenile and adult Dahl's Aquatic frogs are found active along the margins of waterbodies both by day and by night, and thus overlap strongly both in microhabitat and activity times with invasive cane toads (Pizzatto et al. 2008; M.J. Greenlees, *pers. obs.*). These large predatory frogs take both aquatic and terrestrial prey, including the larvae and juveniles of sympatric anurans (Tyler and Cappo 1983). We collected 60 newly metamorphosed Dahl's frogs (mean mass \pm s.d. = 7.15 \pm 2.7 g; range

4.18 to 11.08 g) from Fogg Dam (131°18'48.19"E; 12°34'14.81"S), 60 km southeast of Darwin in the Northern Territory of Australia. Frogs were transported to our laboratory 5 km away. Nightly surveys detected no toad breeding over the preceding months, suggesting that these frogs were naïve to toads. Frogs were housed individually in plastic containers (170 mm long × 120 mm wide × 70 mm high) divided into land plus a pool of water.

On their first night in captivity, all frogs were offered (and consumed) a cricket (*Acheta domesticus*). The following night, 20 individuals were offered a tadpole of a non-toxic native frog (Marbled frog *Limnodynastes convexiusculus*) whereas the remaining 40 were offered a toad tadpole (produced from our captive stock). Tadpoles of both species were at Gosner (1960) developmental stages 25–35 (mean mass ± s.d. of toad tadpoles = 0.05 ± 0.012 g; thus, toad tadpoles constituted 0.5 to 1.1% [mean = 0.7%] of predator mass). Tadpoles were introduced at 1800 h and any regurgitated or surviving tadpoles were removed at 0900 h. One *L. dahlii* died after eating the toad tadpole. On the third night, all frogs that had been exposed to native tadpoles plus 19 of the toad-tadpole-exposed frogs were offered a metamorph cane toad (mean mass ± s.d. = 0.10 ± 0.01 g; thus, prey mass constituted 0.81 to 2.59% [mean = 1.52%] of predator mass). The remaining 20 frogs (all exposed to toad tadpoles the previous night) were given a cricket to test whether exposure to toad tadpoles had affected their readiness to feed.

For each treatment group (native tadpole + toad, toad tadpole + toad, and toad tadpole + cricket) we recorded whether or not the young frog consumed the final prey item (over the course of a trial, from 1800 h to 0900 h), and whether or not the frog survived (as recorded at 0900 h). We used nominal logistic regression to determine whether or not prior exposure to a toad tadpole vs. frog tadpole (the independent variable) affected two dependent variables: (1) whether or not the predator consumed a subsequently-offered metamorph toad, and (2) whether or not a predator survived its encounter with a metamorph toad. All procedures were approved by the University of Sydney Animal Care and Ethics Committee (Approval no. L04/5-2007/3/4479).

Results

As expected from relative toxin content of tadpoles versus terrestrial-stage toads (Hayes et al. 2009), the danger to a naïve anuran predator was greater if its first encounter was with a metamorph toad (6 of 20 died, = 30%) than if its first encounter was with a toad tadpole (1 of 20 died, = 5%; $\chi^2 = 4.72$, $df = 1$, $P = 0.03$) (Figure 1). Most frogs showed no suppression of feeding behaviour the night after consuming a toad tadpole, with 19 of 20 animals consuming crickets that were offered the following night.

Exposure to a toad *versus* frog tadpole did not significantly affect whether or not an adult frog consumed a subsequently-encountered metamorph cane

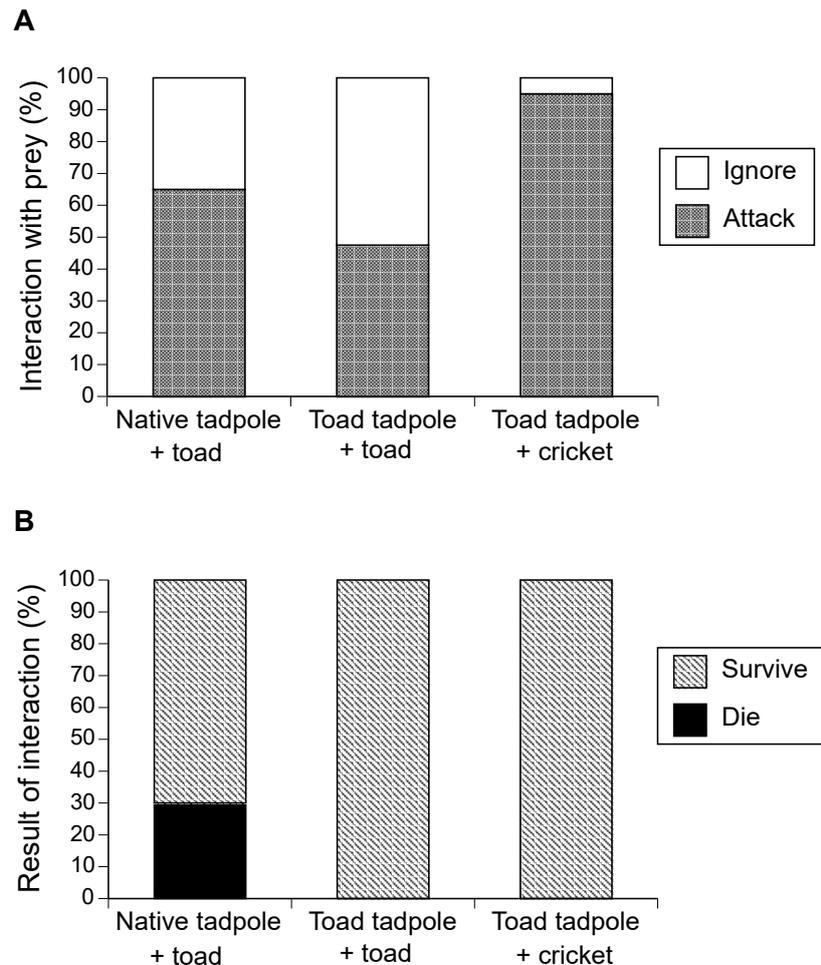


Figure 1. Effect of prior exposure to either a native frog tadpole or toad tadpole on the behaviour and survival rate of Dahl's frogs in laboratory trials. The upper graph (A) summarises rates of ingestion of either a metamorph cane toad (first two bars) or a cricket (third bar) following an initial encounter with either a native frog tadpole (first bar) or a toad tadpole (second and third bars). The lower graph (B) shows the outcome of exposure to these prey types (i.e. survival vs. mortality of the predatory frog). Prior exposure to a (sublethal) toad tadpole did not stop frogs feeding on (non-toxic) crickets, but rendered them less vulnerable in encounters with a (highly toxic) metamorph cane toad.

toad, although the proportion of predators consuming metamorph toads was higher among the frogs previously exposed to native tadpoles (13 of 20, = 65%) than among the frogs initially exposed to toad tadpoles (9 of 19, = 47%; $\chi^2 = 1.628$, $df = 1$, $P = 0.20$) (Figure 1). Importantly, though, six of the 20 frogs that had been exposed only to a native tadpole died when they later encountered a metamorph toad, whereas we recorded no mortality among frogs that had previously consumed a toad tadpole (frog-tadpole-exposed vs. toad-tadpole-exposed, $\chi^2 = 9.38$, $df = 1$, $P = 0.002$) (Figure 1).

Discussion

Our data suggest that attributes of the invasive species (an ontogenetic shift in toxicity: Hayes et al. 2009) and of the native predator (an ability to generalise aversion) play critical roles in the ability of native frogs to survive the invasion of potentially deadly cane toads. Naïve frogs were

more likely to be killed if they encountered a metamorph toad than if they encountered a toad tadpole, but prior experience with a toad tadpole reduced the risk of lethal ingestion during a subsequent encounter with a metamorph. The higher survival rate of “trained” frogs could be attributed to a general lack of interest in food induced by sickness following ingestion of a toxic (albeit non-lethal) tadpole. However, feeding rates on an alternative prey type (crickets) remained high, falsifying this interpretation.

The lower toxicity of toad tadpoles than toad metamorphs to predatory frogs reflects low bufadienolide content at this stage of the life cycle (Hayes et al. 2009). The ability of frogs to learn toad-aversion in response to non-lethal ingestion of toads also has been recorded in adult *L. dahlii* (Shine et al. 2009). However, the ability to generalise aversion from toad tadpoles to toad metamorphs is surprising. The two life-history stages differ in size, colour, shape, locomotor mode and habitat. Ontogenetic consistency in chemical composition (including toxins: Hayes et al. 2009) may provide chemical (taste) cues to predators, such that a frog recognises a similarity between a previously-ingested tadpole and a currently-encountered metamorph. In our trials, frogs apparently responded to that similarity by mouthing but then releasing the metamorph (M. J. Greenlees, *pers. obs.*). This more tentative feeding behaviour may have allowed toxins excreted by metamorphs to be dissipated in water before the metamorph was finally consumed, explaining the between-group difference in frog mortality rates despite a lack of significant difference in consumption rates. Physiological acclimation to the toxin after a single prior exposure is also possible (but has been deemed to be unlikely in previous work: Phillips and Shine 2006).

Although an ability for stimulus generalisation is found across many taxa and through a range of sensory modalities (Ghirlanda and Enquist 2003; Blackiston et al. 2008; Indigo et al. 2018), data for amphibians are limited. These animals are capable of learning via traditional Pavlovian conditioning (Elepfandt 1985; Daneri et al. 2007), but stimulus generalisation has only been demonstrated (in toads) using visual cues in prey identification (Ingle and McKinley 1978). Our results suggest a similar ability acts via chemosensory cues.

Although data are scarce, field observations confirm that *Litoria dahlii* prey upon cane toads around natural waterbodies, and are killed by the toad's toxins (Shine et al. 2009). Hence, the mechanism that we document, whereby exposure to toad tadpoles enhances survival of frogs during subsequent encounters with terrestrial-phase toads, may well be common. Future work could usefully explore related issues, such as the impacts of exposure of larval *L. dahlii* to cane toads of various life stages. More generally, our results suggest an unexpected complexity in the relationship between the invasive species' life-history and its impact on native fauna. All else being equal, the multiphasic life history of the toads would be expected to increase the invader's negative impact because toxic toads can

be encountered in the water (as eggs or tadpoles) or on land (in sizes from < 1 g to > 1 kg; Lever 2001). This broad range of habitats, morphologies, locomotor abilities and sizes renders toxic toads ingestible by a very wide variety of predators (Shine 2010). At the same time, however, individual predators that encounter and respond to smaller, less toxic stages of the toad's life history may thereby be protected from otherwise-fatal encounters with larger and more deadly toads. The multiphasic life history of cane toads thus is a two-edged sword in terms of ecological impact: it increases the number of potentially vulnerable native taxa while simultaneously reducing vulnerability in predator species that consume multiple life-stages of the invasive amphibian.

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