

Embryonic exposure to conspecific chemicals suppresses cane toad growth and survival

Michael R. Crossland and Richard Shine*

School of Biological Sciences, A08, University of Sydney, Sydney, New South Wales 2006, Australia

*Author for correspondence (rick.shine@sydney.edu.au).

Adaptations to suppress the viability of conspecifics may provide novel ways to control invasive taxa. The spread of cane toads (*Rhinella marina*) through tropical Australia has had severe ecological impacts, stimulating a search for biocontrol. Our experiments show that cane toad tadpoles produce waterborne chemical cues that suppress the viability of conspecifics encountering those cues during embryonic development. Brief (72 h) exposure to these cues in the egg and post-hatching phases massively reduced rates of survival and growth of larvae. Body sizes at metamorphosis (about three weeks later) were almost twice as great in control larvae as in tadpole-exposed larvae. The waterborne cue responsible for these effects might provide a weapon to reduce toad recruitment within the species' invaded range.

Keywords: alien species; Anura; *Bufo marinus*; competition; larva; pheromonal communication

1. INTRODUCTION

The damaging ecological impacts of invasive species have spawned a search for novel approaches to control [1,2]. One such opportunity involves the exploitation of species-specific competitive mechanisms, such as pheromones that suppress the reproduction, growth or survival of conspecifics [3–8]. We studied cane toads (*Rhinella marina*), bufonid anurans whose spread through Australia has killed many native predators [9–11]. High densities of toad tadpoles [12,13] result in intense competition, reducing survival, growth and size at metamorphosis [14–17]. Older tadpoles search out and consume eggs before they hatch, thereby reducing competition [17,18]. After they hatch, however, the mobile larvae are invulnerable to attack [17]. If it is difficult for cannibalistic tadpoles to find eggs in muddy weed-choked ponds, we might also expect pheromonal suppression to evolve. Anuran tadpoles use sophisticated chemical communication systems [19] and exhibit plastic developmental responses to chemical cues [20,21]. If toad tadpoles exploit those sensitivities to interfere with their competitors, larval chemical cues might offer novel approaches for targeted control of toads [8,22]. We conducted experiments to look for such effects.

2. MATERIAL AND METHODS

(a) Tadpole husbandry and experimental treatments

We collected adult cane toads from Middle Point Village (12°34' S, 131°18' E) in the Northern Territory, and injected them with

leuprorelin acetate to stimulate oviposition (see [23] for details). A 10-egg section of the egg string was placed into each of 20 containers (17 × 11 × 7 cm; with 750 ml non-chlorinated well water) in a shaded outdoor area. Containers were divided into half with 1 × 1 mm vertical flyscreen mesh. In half of the containers, we added three cane toad tadpoles collected from a local pond (snout–vent length (SVL) 8.75–10.15 mm; Gosner stage 34–36 [24]), to the opposite side of the mesh (no food was provided). Thus, eggs were exposed to waterborne cues from tadpoles, but no physical contact. The remaining 10 containers (randomly allocated) served as controls (no tadpoles).

After 72 h, when the eggs had developed into free-swimming tadpoles (Gosner stage 25), we tested water quality (dissolved oxygen and temperature using a YSI 85 meter (Yellow Springs, OH, USA); ammonia and pH using API test kits (Chalfont, PA, USA) and SSS Universal Indicator Paper (Murarrie, Queensland)). Each group of 10 newly hatched tadpoles was then transferred to a larger container (37 × 28 × 20 cm; 20 tubs total) containing a 2 cm deep sediment from a nearby pond, and filled to a depth of 15 cm with non-chlorinated water, and 1 g Hikari algae pellets (Kyorin Co. Ltd., Himeji City, Japan) per tub to provide additional nutrients.

Five days later, we randomly euthanased all tadpoles in five replicates per treatment (with MS-222; Argent Chemicals, Redmond, WA, USA), and counted and measured them (SVL, mass after blotting dry, Gosner stage). The remaining tadpoles were checked daily, and placed individually in moist paper-lined containers when they began to metamorphose. When the limbs had emerged and the tail was resorbed, metamorphs were measured (snout–urostyle length (SUL), blotted dry mass) and the length of the larval period was calculated.

(b) Data analyses

Data were analysed using MANOVA and *t*-tests, using mean values per container to avoid pseudoreplication. If necessary, data were log-transformed to assure normality and variance homogeneity. Survival data were arcsine transformed. Non-normally distributed ammonia data were analysed using the Kruskal–Wallis test.

3. RESULTS

The two treatments did not differ in mean dissolved oxygen concentration (control = 6.91 mg ml⁻¹, conspecific exposure = 6.85 mg ml⁻¹; $t_{18} = 1.04$, $p = 0.31$), temperature (27.1°C, 26.7°C; $t_{18} = 1.78$, $p = 0.09$) or pH (6.0 in all containers), but ammonia concentration increased (0.5, 1.0 mg ml⁻¹; $\chi^2_1 = 19.0$, $p < 0.0001$).

Exposure to chemical cues from conspecific tadpoles reduced the viability of larvae hatching from those eggs, at 5 days post-hatching (figure 1; MANOVA, $F_{4,5} = 12.45$, $p < 0.001$) and at metamorphosis (figure 2; MANOVA, $F_{4,5} = 5.19$, $p < 0.05$). When assessed after 5 days, exposure did not affect survival rate ($t_8 = 0.79$, $p = 0.45$), but reduced the treatment tadpoles' body length (24% decrease, $t_8 = 4.06$, $p < 0.005$), body mass (41% decrease, $t_8 = 3.40$, $p < 0.01$) and developmental stage ($t_8 = 5.43$, $p < 0.001$). The effects of embryonic exposure were evident more than 20 days later (figure 2). Larvae from tadpole-exposed eggs took greater than 8 per cent longer to complete development, but this difference was not significant ($t_8 = 1.20$, $p = 0.26$). Metamorphs from the tadpole-exposure treatment averaged 11 per cent shorter ($t_8 = 2.270$, $p < 0.03$) and 45 per cent lighter ($t_8 = 2.79$, $p < 0.03$) than did unexposed siblings, and their survival rates were reduced by 40 per cent ($t_8 = 3.24$, $p < 0.05$; figure 2).

4. DISCUSSION

Embryonic exposure to chemical cues from cane toad tadpoles had devastating long-term consequences for conspecific larvae, even though water quality remained well within the range tolerated by cane toad tadpoles [25]. Larval survival, growth and development were

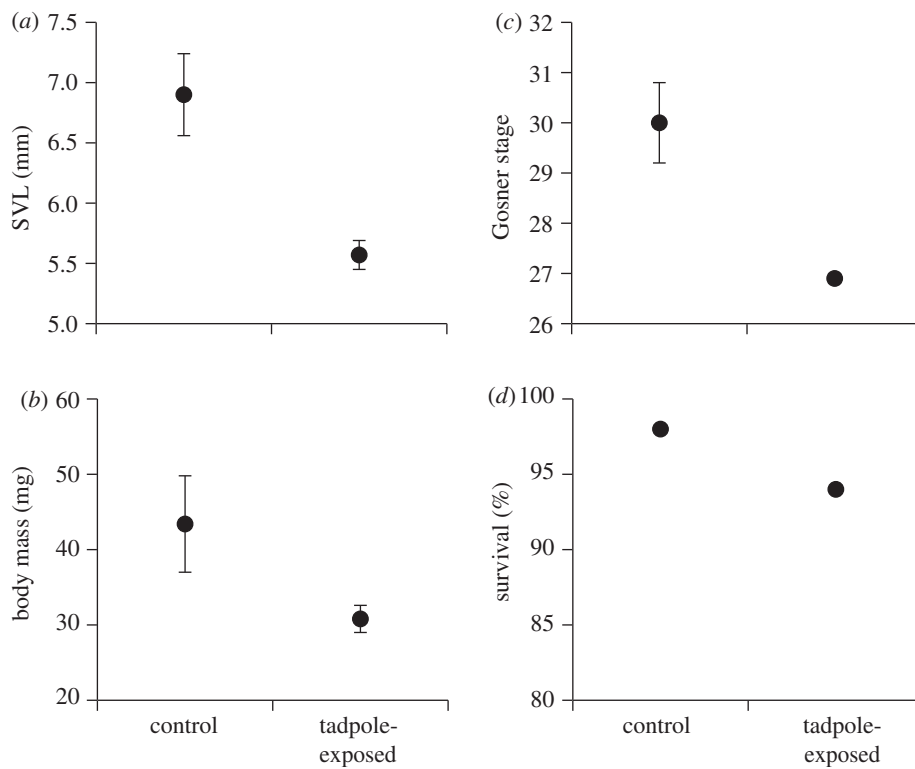


Figure 1. Phenotypic traits of cane toad tadpoles at 5 days post-hatching, as a function of exposure to chemicals from conspecific tadpoles during the egg stage. These chemicals reduced the younger tadpoles' (a) body length, (b) mass and (c) developmental stage, but (d) survival rates were unaffected. Graphs show means and standard errors.

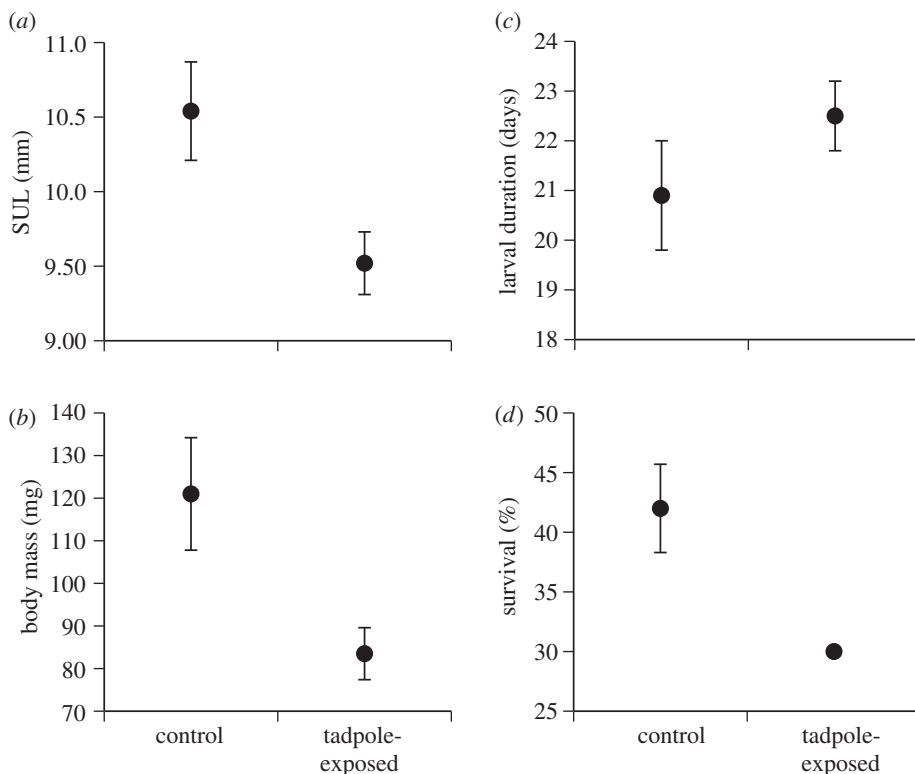


Figure 2. Phenotypic traits of metamorph cane toads as a function of exposure to chemical cues from conspecific tadpoles during the egg stage. Exposure to these cues reduced the (a) body length and (b) mass of metamorphs, (c) non-significantly extended the duration of the larval stage and (d) reduced larval survival. Graphs show means and standard errors.

substantially reduced, with metamorphosis at smaller body sizes. Delayed metamorphosis can impose a heavy fitness cost [26], and smaller tadpoles are more vulnerable to predation [27] and competition [28,29]. Smaller metamorphs are more vulnerable to

desiccation [30], predation [31], cannibalism [32] and parasitism [33].

Bufo tadpoles possess specialized epidermal secretory cells ('riesenzellen') that produce pheromones [34–36], possibly including these suppressors

of the viability of conspecific larvae. Pheromonal production by the older tadpoles may be continuous, or may have been evoked by the presence of eggs (tadpoles can detect eggs from waterborne cues [17,18]) or by starvation [37]. These possibilities could be tested by exposing eggs to water from tadpoles that had or had not been exposed previously to conspecific eggs, or from fed versus unfed tadpoles. Chemical suppression of embryos might be owing to an adaptation of tadpoles, an adaptive plastic response of embryos and/or a fortuitous (unselected) effect. Future work could usefully explore these alternatives, as well as assessing the consistency of this response across pond conditions, sibships and the like.

Unlike other toad pheromones [18,35], these effects reduce toad viability in the long term only after brief exposure. Thus, they have great potential as a species-specific pheromonal control for invasive cane toads. Australian native anurans do not respond to the alarm or attractant pheromones produced by cane toad tadpoles [18,38], and so may well ignore these development-suppressing toad pheromones. Sex pheromones have been used as attractants and mating disruptors for biocontrol [6,7,39]. Agricultural scientists exploit allelopathic effects to suppress weed growth [40]; and indeed, the effects we have documented would qualify as allelopathy under some but not all definitions of this term [41]. In a similar vein, anuran pheromones that reduce the viability of conspecifics may provide powerful weapons for the control of selected species.

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