Embryonic exposure to conspecific chemicals suppresses cane toad growth and survival

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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 conspecífics [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 leuprolin 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 1.5 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 the 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 euthanized 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; t₁₈ = 1.04, p = 0.31), temperature (27.1°C, 26.7°C; t₁₈ = 1.78, p = 0.09) or pH (6.0 in all containers), but ammonia concentration increased (0.5, 1.0 mg ml⁻¹; X² = 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, F₄.₅ = 12.45, p < 0.001) and at metamorphosis (figure 2; MANOVA, F₄.₅ = 5.19, p < 0.05). When assessed after 5 days, exposure did not affect survival rate (t₆ = 0.79, p = 0.45), but reduced the treatment tadpoles’ body length (24% decrease, t₆ = 4.06, p < 0.005), body mass (41% decrease, t₆ = 3.40, p < 0.01) and developmental stage (t₆ = 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 (t₆ = 1.20, p = 0.26). Metamorphs from the tadpole-exposure treatment averaged 11 per cent shorter (t₆ = 2.270, p < 0.03) and 45 per cent lighter (t₆ = 2.79, p < 0.03) than did unexposed siblings, and their survival rates were reduced by 40 per cent (t₆ = 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
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].

Bufonid tadpoles possess specialized epidermal secretory cells (‘riesenzellen’) that produce pheromones [34–36], possibly including these suppressors.
of the viability of conspecific larvae. Phenomeral pro-
duction by the older tadpoles may be continuous, or
may have been evoked by the presence of eggs (tad-
poles 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 sup-
pression of embryos might be owing to an adaptation
to 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
specie-specific phenomeral 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 documen-
ted would qualify as allelopathy under some but not all
promotions for management.

The work was approved by the University of Sydney Animal
Ethics Committee.

We thank Michelle Franklin and Nilusha Somaweera for
assistance, and the Australian Research Council for funding.

Environmental and economic costs of nonindigenous
1641/0006-3568(2000)050[0053:ECAON]2.3.CO;2)

2 Blaustein, A. R. & Kiesecker, J. M. 2002 Complexity in
conservation: lessons from the global decline of amphi-
j.1461-0248.2002.00352.x)

3 Drickamer, L. C. 1984 Urinary chemosignals from mice
(Mus musculus): acceleration and delay of puberty in
related and unrelated young females. J. Comp. Physiol.
98, 421–431.

4 Gerlach, G. 2006 Phenomeral regulation of reproductive
success in female zebrafish: female suppression and male
1016/j.anbehav.2006.03.009)

5 Marsh, R. E. & Howard, W. E. 1979 Pheromones (odors)

6 Thomson, D. R., Gut, L. J. & Jenkins, J. W. 1999 Pher-
onmes for insect control: strategies and successes.

7 Li, W., Siefkes, M. J., Scott, A. P. & Teeter, J. H. 2002 Sex
pheromone communication in the sea lamprey: implica-
tions for management. J. Ft Lakes Res. 29(Suppl. 1),
85–94. (doi:10.1016/S0380-1330(03)70479-1)

8 Wassersug, R. J. 1997 Assessing and controlling amphi-
bion populations from the larval perspective. In
Amphibians in decline: Canadian studies of a global problem
(ed. D. Green), pp. 271–281 (Herpetological conserva-
tion no. 1). Saint Louis, MO: Society for the Study of
Amphibians and Reptiles.

9 Lever, C. 2001 The cane toad. The history and ecology of a
successful colonist. Otley, UK: Westbury Scientific
Publishing.

10 Kraus, F. 2009 Alien reptiles and amphibians: a scientific
compendium and analysis. Dordrecht, The Netherlands:
Springer Science and Business Media BV.

11 Shine, R. 2010 The ecological impact of invasive cane
toads (Bufo marinus) in Australia. Q. Rev. Biol. 85,
253–291. (doi:10.1086/655116)

12 Alford, R. A., Cohen, M. P., Crossland, M. R.,
Hearden, M. N. & Schwarzkopf, L. 1995 Population
biology of Bufo marinus in northern Australia. In
Wetland research in the wet–dry tropics of Australia (ed. C. M.
Finlayson), pp. 173–181 (Supervising Scientist Report
101). Canberra, Australia: Supervising Scientist
Commonwealth of Australia.

13 Williamson, I. 1999 Competition between the larvae of
the introduced cane toad Bufo marinus (Anura: Bufoni-
dae) and native anurans from the Darling Downs area
(doi:10.1046/j.1442-9933.1999.00993.x)

14 Alford, R. A. 1994 Interference and exploitation competi-
tion in larval Bufo marinus. In Advances in ecology and
environmental studies (eds P. C. Mishra, N. Behara, B. K.
Senapati & B. C. Guru), pp. 297–306. New Dehli, India:
Ashish Publishing House.

of the invasive cane toad (Bufo marinus) on an Australian
frog (Opisthodon ornatus) depends on reproductive timing.
Oecologia 158, 625–632. (doi:10.1007/s00442-
008-1167-y)

16 Cabrera-Guzmán, E., Crossland, M. R. & Shine, R.
2011 Can we use the tadpoles of Australian frogs to

17 Crossland, M. R., Hearden, M. N., Pizzatto, L., Alford,
R. A. & Shine, R. In press. Why be a cannibal? The
benefits to cane toad, Rhinella marina [=Bufo marinus],
tadpoles of consuming conspecific eggs. Anim. Behav.

18 Crossland, M. R. & Shine, R. 2011 Cues for cannibalism:
 cane toad tadpoles use chemical signals to locate and
consume conspecific eggs. Oikos 120, 327–332.
(doi:10.1111/j.1600-0706.2010.18911.x)

19 Waldman, B. & Bishop, P. J. 2004 Chemical communi-
cation in an archaic anuran amphibian. Behav. Ecol. 15,

20 Benard, M. F. 2004 Predator-induced phenotypic plas-
ticity in organisms with complex life-histories. Annu.
anurev.ecolsys.35.021004.112426)

21 Touchon, J. C. & Wardent, K. M. 2008 Fish and dra-
gonfly nymph predators induce opposite shifts in color
and morphology of tadpoles. Oikos 117, 634–640.

22 Faragher, S. G. & Jurgler, R. G. 1998 Tadpole bullies:
examining mechanisms of competition in a community
of larval anurans. Can. J. Zool. 76, 144–153. (doi:
10.1139/z97-177)

23 Hayes, R. A., Crossland, M. R., Hagman, M., Capon,
R. J. & Shine, R. 2009 Ontogenetic variation in the
chemical defenses of cane toads (Bufo marinus): toxin
profiles and effect on predators. J. Chem. Ecol. 35,

24 Gosner, K. L. 1960 A simplified table for staging anuran
embryos and larvae with notes on identification. Herpeto-
lologia 16, 183–190.
Intraspecific suppression for biocontrol

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