Pheromones can cull an invasive amphibian without releasing survivors from intraspecific competition

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Abstract. Attempts to cull an invasive species often create a paradoxical situation, whereby the consequent reduction in invader densities frees the survivors from intraspecific competition—and hence, increases the viability of those survivors. Our laboratory experiments with invasive cane toads (Rhinella marina) show that this backfire effect can occur with pheromone-baited trapping. Eliminating most of the tadpoles from a tank accelerates metamorphosis of the survivors and increases the size (and thus quality) of those metamorphs. Thus, trapping is likely to reduce recruitment only if the program catches all, or almost all, of the tadpoles in a waterbody. In contrast, toad control using the suppression pheromone, either alone or alongside trapping, causes similar rates of mortality as via trapping alone, but the survivors exhibit smaller mass at metamorphosis and a longer, not shorter, larval period. Thus, the combination of pheromone-based suppression and trapping can reduce effective recruitment of cane toads more successfully than can either method when used alone.

Key words: biocontrol; Bufo marinus; density dependence; invasive species; pheromones.

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INTRODUCTION

The number of species that have expanded their geographic ranges due to human involvement has risen dramatically over the past 200 yr, largely due to a marked increase in global transport and commerce (di Castri 1989, Mack et al. 2000, Mooney and Cleland 2001). In most cases, organisms that are transported to areas far outside their native range will perish en route, or be unable to survive in a new environment outside the species’ fundamental niche (Mack et al. 2000). In a few cases, however, organisms are able to proliferate, spread, and persist, creating a range of ecological problems in the newly invaded environment (Vitousek et al. 1996). Thus, invasive species control is of high priority to wildlife managers, and the development of more effective control measures is vital to conservation biology (D’antonio and Kark 2002).

The history of wildlife management underscores the difficulty of eradicating invasive species (e.g., Saunders et al. 2015). Nonetheless, in some cases control efforts have prevented substantial ecological damage (Mack et al. 2000). For example, culling has eradicated feral goats from 120 islands worldwide (Campbell and Donlan 2005), and removal of the long-spined sea urchin (Centrostephanus rodgersii) along the Tasmanian coast of Australia has substantially reduced its density, as well as the occurrence of urchin barrens in treated areas (Tracey et al. 2015). Failed control attempts often reflect a lack of understanding of a species’ biology. For example, shy–bold personality traits of individuals can affect their trappability (Mills and Faure 2000, Carter...
et al. 2012), such that trapping schemes lower short-term population densities but simultaneously impose strong selection on behavioral traits that render the surviving individuals and their progeny far less vulnerable to this form of control. Similarly, targeting particular life stages of an invasive population can have unexpected results due to complex underlying population dynamics. For example, when soil miles (Sancassania berlesei) were selectively removed from a population in their egg stage, the overall survival and consequent population size actually increased due to the lowered intraspecific competition between the surviving individuals (Benton et al. 2004). This kind of density dependence is common: That is, per-individual fitness is higher at lower densities, due to an increase in available resources (De Roos et al. 2007, Smith et al. 2017). Thus, control efforts need to take into consideration the complex effects of removing individuals from an invasive population, before such strategies are deployed.

One successful invasive species is the cane toad, Rhinella marina (Lowe et al. 2000). Although native to South and Central America, these large bufonid anurans have been introduced to more than 40 countries globally; they have been present in Australia since 1935 (Shine 2010). In this time, cane toads have spread rapidly westward and southward from their initial invasion point in northeastern Queensland, and currently can be found over more than 1.2 million km² of tropical and subtropical Australia (Urban et al. 2008). Cane toads have had devastating impacts on Australian wildlife, largely due to the vulnerability of frog-consuming predators to the toads’ powerful toxins (Shine 2010). As toads continue to spread further into western and southern Australia, we urgently need to develop management strategies, in order to reduce the impact of toads on native fauna.

To date, efforts to control cane toad numbers in Australia have been largely unsuccessful (e.g., manual removal, Boulter et al. 2006; terrestrial traps, Schwarzkopf and Alford 2006), but recent work has revealed the potential for a new avenue of control—manipulating the chemical cues used by cane toad tadpoles (Crossland et al. 2012, Clarke et al. 2015, Tingley et al. 2017). Two new methodologies show great promise. Firstly, traps that exploit the chemical attraction of toad tadpoles to conspecific eggs (a response that evolved in the context of cannibalism) can be used to remove toad tadpoles from waterbodies (Crossland and Shine 2011). The attractive cue emitted by toad eggs is mimicked using bufotoxin squeezed from adult toads. Funnel traps that contain this toxin capture toad tadpoles without attracting native fauna (Crossland et al. 2012). Although this method can capture large numbers of tadpoles from natural waterbodies (Crossland et al. 2012), the consequences of that removal for total effective recruitment remain unclear. Anuran tadpoles generally have low survival due to intense competition, creating a strong density dependence such that any reduction in density (as can be achieved by trapping) benefits the surviving individuals (Alford et al. 1999). Potentially, then, eliminating half or three-quarters of the toad tadpoles in a pond might increase rather than decrease the number of metamorphs that eventually survive and disperse away from that pond into the surrounding landscape. Clearly, such a feedback system could be catastrophic for the use of toxin-baited traps for toad control.

The second new method involves the suppression of toad eggs and hatchlings by older conspecific tadpoles (Crossland and Shine 2011). Toad hatchlings that are exposed to chemical cues (the suppression cue) from older conspecific tadpoles exhibit a dramatic decrease in both survival and growth during the larval phase (Clarke et al. 2015, 2016). Although this strategy works well under laboratory conditions, many questions remain unanswered. Notably, will a reduction in tadpole density (due to the mortality induced by suppression) release the survivors from intraspecific competition, in the same way as suggested above for trapping? Additionally, the toad tadpoles that develop from suppressor-exposed eggs tend to be less easily trappable, because they show a less intense attraction to bufotoxins (S. McCann, unpublished data). Such an effect might render trapping and use of the suppression pheromone incompatible as control methods.

Our experiments explored the impact of these two control methods on the survival and fitness of toad tadpoles under field-like conditions. Specifically, how do tadpole trapping and suppression influence the fate of surviving individuals, and how might these treatments interact?
**METHODS**

**Toad collection and breeding**

Adult cane toads were collected by hand from Doongan in Western Australia (WA –15.390413, 126.293106) and brought back to our laboratory at Middle Point, Northern Territory (NT –12.579564, 131.313918). Within two weeks of collection, pairs of adult toads were injected with leuprolerelin acetate (Lucrin, Abbott Australasia) to induce breeding (see Hayes et al. 2009 for detailed methods). Newly laid clutches of eggs were kept in individual tubs (18 L capacity) in unchlorinated water at room temperature (25°C) and aerated until they reached Gosner stage 18 (hatched but immobile, approximately 36 h after eggs were laid, Gosner 1960).

**Mesocosms**

Seventy-two plastic aquaria (590 x 360 x 380 mm) were set up in a shaded outdoor area and lined with dry soil (1 L) collected from a nearby paddock. Unchlorinated water (60 L) and crushed algal wafers (1 g) were added to each tub and left to settle for 6 d.

**Suppression treatment**

We allocated aquaria to one of three experimental treatments: control, three-tadpole suppression, or thirty-tadpole suppression, with 24 aquaria per treatment. We placed a flyscreen mesh container (holes 1 x 1 mm, container 150 x 80 x 150 mm) into the water in each aquarium and lined with dry soil (1 L) collected from a nearby paddock. Unchlorinated water (60 L) and crushed algal wafers (1 g) were added to each tub and left to settle for 6 d.

Twelve hours later, the Doongan (WA) experimental hatchlings at Gosner stage 18 were randomly allocated, 20 to each aquarium (three clutches, 24 tanks each). We placed the hatchlings into a plastic container (1 L capacity) with flyscreen sides (holes 1 x 1 mm) and floated it near the top of the aquarium to ensure that hatchlings were exposed to cues in the water. We added 0.5 g of crushed alga wafers to each of the experimental aquaria (regardless of tadpole density) on days 8, 16, 40, and 69, to ensure food availability. The number of living tadpoles in each aquarium was recorded every second day for the duration of the experiment.

**Trapping treatment**

On day 11, we allocated half of each of the suppression treatment aquaria to toxin trapping and the other half to controls. Tadpole traps were transparent 1-L containers containing an inward-pointing funnel (length 35 mm, diameter 44 mm, smallest diameter of funnel 12 mm) with one trap per toxin trapping tub secured to the side of the aquarium near the water surface. Toxin baits (attractant cues) were made by squeezing 200 mg of fresh toxin from the parotid glands of adult female toads into a cup filled with 30 mL of unchlorinated water. Toxin from each adult female toad was used to make between 3 and 6 baits, and these were randomly allocated across clutches and treatment groups. The baits were left to sit for 30 min at room temperature before being added to the traps. The numbers of tadpoles inside each trap were counted 90 min after adding the bait, after which the traps were removed, and the captured tadpoles weighed (g) and euthanized. This procedure was repeated on day 15, in the same aquaria as the previous trapping treatment. Pilot studies indicated that toxin baits remained attractive for approximately 90 min.

**Metamorphosis**

On day 20, we added a floating platform (5 cm diameter) with attached flyscreen to each experimental aquarium, to allow metamorphosing tadpoles to rest at the water surface. When the front legs of a tadpole started to emerge, we placed the animal into an individual plastic cup (70 mL) with 10 mL of tub water and floated it in its aquarium until its tail was completely reabsorbed. We recorded the total days taken until metamorphosis (complete reabsorption of tail), and the metamorph was then weighed (g).

The experiment was terminated at day 100, as the few remaining tadpoles could be presumed...
ecologically dead due to the high likelihood that dropping water levels or aquatic predators would have led to their death under field conditions.

**Data analysis**

Initial analyses showed that the effect of the two densities of tadpoles used for suppression (3 vs. 30) was statistically indistinguishable (no significant main effects or interaction terms). Thus, we combined tadpoles exposed to these two treatments into a single category (suppressed).

We used logistic regression in R v3.3.3 (R Core Team 2014, see Warton and Hui 2011) to compare the proportion of tadpoles trapped over two trapping sessions in suppressed vs. control tubs. We also used logistic regression in R to compare the proportion of tadpoles surviving to metamorphosis using suppression treatment, trapping treatment, and suppression treatment × trapping treatment as fixed effects. This analysis was based on the quasibinomial distribution to account for over-dispersion of data.

We used 2-way ANOVAs in SPSS v22 (IBM, Armonk, New York, USA) to compare the mass and days to metamorphosis, using suppression treatment and trapping treatment as fixed factors. Clutch of the tadpoles tested was included in all analyses as a random factor in the model.

**RESULTS**

**Trappability**

The toxin-baited traps caught more tadpoles from control tanks than from tanks containing tadpoles that had previously been exposed to the suppression cue, regardless of suppressor density (\( t = 2.72, df = 1, P = 0.01 \); Fig. 1).

**Survival**

The mean survival of tadpoles to metamorphosis ranged from approximately 15% (trapping treatments) to 50% (control, no trapping), and was influenced by a significant interaction between suppression treatment and trapping treatment (interaction, \( t = 2.62, df = 1, P = 0.01 \); Fig. 2). In the absence of trapping, survival rates were lower for suppressed tadpoles than for control tadpoles; but if trapping was added, the overall survival rates of the two groups were similarly low (Fig. 2). That is, trapping reduced...
survival of suppressed tadpoles less than non-suppressed tadpoles, such that their final overall survival rates were similar (Fig. 2).

**Tadpole densities**

The number of tadpoles present in each tub (i.e., tadpole density) decreased rapidly in tubs that were trapped on days 11 and 15, particularly in the non-suppressed treatment group (Fig. 3). In tubs that were not trapped, densities dropped gradually over time. After day 22, densities in all treatments fell due to a combination of deaths and tadpoles reaching metamorphosis.

**Mass of metamorphs**

The mean mass of metamorphs was influenced by an interaction between suppression treatment and trapping treatment (ANOVA, $F_{1,68} = 5.23$, $P = 0.03$). Metamorphs from tubs that were trapped were heavier than those from tubs that were not trapped (Fig. 4). In tubs that were not trapped, metamorphs from suppression-treated tadpoles were heavier than metamorphs from control tubs (Fig. 4). To test whether this effect was mediated by the density of tadpoles present in each tub, we reran our analysis to include the number of tadpoles present in each tub on day 21 of the experiment as an additional covariate. Day 21 was chosen, as this was the day prior to the first tadpole metamorphosing in the experiment; densities at this time reflect the conditions influencing metamorphosing tadpoles. When tadpole counts from day 21 were included in the analysis, there was no longer an effect of either suppression or trapping on the mass of metamorphs (suppression, $F_{1,68} = 0.25$, $P = 0.62$; trapping, $F_{1,68} = 0.09$, $P = 0.77$; suppression $\times$ trapping, $F_{1,68} = 1.95$, $P = 0.17$), whereas the effect of tadpole counts was significant ($P = 0.004$). Thus, a reduction in tadpole density (i.e., lower survival, due either to trapping or to suppression) appears to increase size at metamorphosis of the survivors.

**Days to metamorphosis**

The number of days taken for tadpoles to metamorphose was influenced by an interaction between suppression treatment and trapping.
treatment (ANOVA interaction; $F_{1,68} = 4.53, P = 0.04$; Fig. 5). When tadpoles were not suppressed, they metamorphosed faster when trapping had occurred, compared to tubs that had not been trapped (approximately 29 vs. 35 days, respectively). However, suppressed tadpoles took longer to metamorphose, regardless of trapping treatments (38–40 d on average).

**DISCUSSION**

Our results confirm that removing toad tadpoles from a waterbody, although reducing overall survival, creates larger metamorphs than would have been the case if trapping did not occur. This effect is due to the decrease in tadpole density caused by trapping and the consequent increase in resources available per individual (i.e., density dependence: Alford et al. 1999). Larger cane toad metamorphs have better locomotor performance, are better able to catch and consume prey, and grow and survive better than do smaller individuals (Cabrera-Guzmán et al. 2013). Thus, trapping from ponds increases the quality of surviving metamorphs. Treatment with the suppression cue, however, resulted in a similar reduction in survival of tadpoles to trapping, while only minimally increasing the size of surviving individuals. Unlike trapping, pheromonal suppression produces surviving but slow-growing tadpoles that are unlikely to survive to metamorphosis (Clarke et al. 2015); such tadpoles thus remain in the waterbody (and compete with conspecifics) rather than being eliminated (as would occur with trapping). Hence, suppression treatment has little effect on the quality of surviving metamorphs.

Both trapping and suppression treatments affected the time taken for surviving tadpoles to reach metamorphosis. Trapping accelerated metamorphosis, whereas treatment with
suppression cue (whether combined with trapping or not) delayed it. Faster time to metamorphosis is an impediment to control efforts, as it reduces the period of natural suppression (i.e., when tadpoles from earlier clutches are present in a waterbody), allowing for new egg clutches to be laid without negative suppression effects. Early attainment of metamorphosis also reduces the period when tadpoles are accessible to trapping. Collateral costs of longer larval life of the pest species to native wildlife are low; the tadpole is the least toxic stage of the cane toad life cycle, and predation on toad tadpoles is not a major threat to native anurans (Crossland and Alford 1998, Hayes et al. 2009). Thus, by extending the duration of the tadpole period, treatment with suppression cue prolongs the window of time that eggs laid in that pond would be suppressed, without exacerbating the effect of toad larval presence on native wildlife.

Despite the negative consequences of trapping toad tadpoles (higher quality and faster metamorphosis of survivors), trapping may be worthwhile under some circumstances. For example, tadpole trapping under ideal conditions (e.g., in a small, clear, and isolated waterbody) can be 100% effective, removing all toad tadpoles from a focal waterbody without leaving any survivors (Crossland et al. 2012). In this case, trapping provides a fast and effective solution. Further studies could usefully determine which biotic or abiotic factors influence capture rates during trapping, to identify when and where trapping can be most successful.

Our results indicate that applying the suppression cue to waterbodies can reduce survival of cane toad tadpoles, while also extending their time to metamorphosis (and hence, ensuring that subsequent clutches are suppressed). Unfortunately, the current protocol for providing the suppression cue—placing live tadpoles inside a net within a waterbody (S. McCann, unpublished data)—is not easily applicable under most circumstances. Researchers at the University of Sydney and the University of Queensland are currently attempting to isolate and identify the chemical (or suite of chemicals) that causes the suppression effect, in order to find a more practical way of applying the suppression cue in the field (M. Crossland, personal communication). If this is successful, the cue could be applied to waterbodies during periods of breeding (e.g., after rain), to reduce cane toad recruitment without impacting native fauna.

Finally, we suggest that this experiment be repeated under field conditions in order to confirm that the patterns we see in this study persist under natural densities, predation, temperature, and weather fluctuations. Such factors might well affect outcomes. For example, suppressed tadpoles are more susceptible to predation by gape-limited predators than are non-suppressed individuals, due to their smaller size (S. McCann, unpublished data). Thus, suppression of tadpoles in the field may induce a further decrease in larval survival via higher predation. The consequent reduction in tadpole densities within suppression-treated populations may in turn influence the fitness of survivors.

Although it is unlikely that cane toads will be eradicated from Australia, reducing toad recruitment nonetheless may decrease the impact of toads on native fauna. Control may be especially effective in isolated extralimital sites. Toads frequently hitchhike to locations far from the invasion front, as stowaways in trucks or campervans (White and Shine 2009). Although such toads usually perish, translocation of multiple individuals can result in an isolated breeding population, imperiling naive wildlife. This situation occurred in southern Sydney in 2010, with successful breeding and over 500 individuals removed over the following two years (M. Greenlees et al., unpublished data). Dealing with such incursions would benefit from effective and efficient methods of toad management that could be immediately rolled out when a breeding population is discovered. Other high-priority situations include the invasion of small islands or areas of high conservation value, and locations where the toad invasion front is progressing slowly (e.g., the southern invasion front in northern New South Wales: Kolbe et al. 2010, McCann et al. 2014).

Understanding the biology of a pest species can help us to minimize the negative consequences of control efforts—in this case, by ensuring that culling does not improve the viability of survivors. Using the tadpole suppression treatment reduced the larval population’s ability to bounce back after trapping, by removing the benefits of a reduction in tadpole density for the remaining individuals. More generally, optimal
control methods for invasive species often may be multifaceted, exploiting a range of responses to maximize the impact of control efforts on the target population. For example, invasive plant management often combines chemical, biological, and physical control methods with habitat manipulation, modification of cultural practices, and use of resistant varieties (DiTomaso et al. 2017). By utilizing strategy-specific recommendations, and incorporating a range of management strategies, land managers can enhance their capacity to manage the invasive cane toad.

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LITERATURE CITED


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