

# Rapidly evolved traits enable new conservation tools: perspectives from the cane toad invasion of Australia

Richard Shine<sup>1</sup>  and Simon Baeckens<sup>2,3</sup> 

<sup>1</sup>School of Natural Sciences, Macquarie University, Sydney, Australia

<sup>2</sup>Department of Biology, University of Antwerp, Antwerp, Belgium

<sup>3</sup>Department of Biology, Ghent University, Ghent, Belgium

Corresponding author: School of Natural Sciences, Macquarie University, Sydney, Australia. Email: [rick.shine@mq.edu.au](mailto:rick.shine@mq.edu.au)

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## Abstract

Natural populations can show rapid adaptive responses to intense (human-mediated) environmental change. The potential for exploiting rapidly evolved traits for conservation management has been often discussed but rarely implemented. Capitalizing on a well-studied biological invasion, we here explore the idea that rapid phenotypic change in the invaders, their pathogens, and the native biota provide opportunities for managers to control invader abundance and buffer adverse impacts on native wildlife. Intensive studies of the invasion of tropical Australia by cane toads (*Rhinella marina*) have identified newly evolved vulnerabilities that we could exploit for toad control; and newly evolved resilience of native wildlife that we could exploit for impact reduction. For example, distinctive phenotypes of toads at the expanding range edge enhance dispersal rate but reduce reproductive output, intraspecific competitive ability, and immunocompetence; and the evolution of larval cannibalism creates opportunities not only for species-specific trapping of toad tadpoles, but also could be exploited (when allied to emerging CRISPR-Cas9 techniques) to intensify intraspecific conflict in invasive toads. That is, we could use the invasive species to control their own populations. This case study illustrates the potential of detailed basic research to identify novel approaches for conservation.

**Keywords:** biological conservation, contemporary evolution, invasion biology, *Bufo marinus*

## Introduction

The long-standing view that evolutionary change occurs slowly, over long periods of time, has come under strong challenge (Carroll et al., 2007; Hairston et al., 2005; Reznick et al., 2019). Numerous studies have documented rapid evolutionary shifts in response to pollution (e.g., Reid et al., 2016), overharvesting (e.g., Coltman et al., 2003; Niu et al., 2021), climate change (e.g., Donihue et al., 2018; Geerts et al., 2015), urbanization (e.g., Santangelo et al., 2022; Winchell et al., 2023), and the invasion of non-native organisms (e.g., Phillips & Shine, 2006; Stuart et al., 2014). Such processes can induce sudden changes in the biotic or abiotic conditions experienced by populations, causing a rapid shift in the way in which individual fitness maps onto phenotypic variation (Reznick & Ghalambor, 2001). As a result, phenotypes adaptive to the ancestral situation become maladaptive. The outcome of such sudden directional selection can be rapid evolutionary change.

The potential for rapid evolutionary adjustments is a double-edged sword for conservation managers. The positive side of the equation is that if we can maintain large genetically diverse populations of a threatened species, adaptive changes may increase the resilience of that taxon to any new challenges. Even without directly addressing the mechanism of impact, maintaining a population's adaptive potential and

ability to evolve can foster long-term viability (Gomulkiewicz & Holt, 1995). The negative side is that rapid evolution in an invasive species may exacerbate management problems as the invader evolves to better exploit the new opportunities in its invaded range, or to circumvent attempts at control.

Can managers move beyond simply providing opportunities for adaptation to novel challenges, and use an understanding of evolutionary change to frame novel interventions? That option has been often discussed but rarely implemented (Kinnison et al., 2007; Stockwell et al., 2003), for two main reasons. One is a concern that Darwinian approaches might backfire, creating unforeseen problems such as unintended introduction of pathogens with translocated hosts (Cunningham, 1996). The extensive paperwork required to approve translocations also discourages this approach (Berger-Tal et al., 2020). Another obstacle centers on widespread reluctance to embrace genetic editing techniques (such as CRISPR-Cas9) either because of doubts about long-term impacts (e.g., unforeseen transfer of genetically modified organism [GMO] material to non-target species), or extensive regulatory impediments to release of GMOs (Love Stowell et al., 2017; Muir & Howard, 1999). A final reason is that the eco-evolutionary dynamics of only a few natural systems have been studied in enough detail (Hendry, 2017, 2019), reducing confidence in our ability to predict outcomes of interventions. Below, we briefly review published ideas about evolutionary

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approaches to wildlife management, then discuss a well-studied system (invasive cane toads in Australia) in detail. Specifically, we review traits that have evolved rapidly during the toad invasion, creating novel vulnerabilities that could be exploited for management of invasive toads.

## Evolution-focused management approaches

Global climate change has led to increased sea water temperatures and modified ocean chemistry. Many corals are at risk and the loss of coral will have detrimental consequences to life on earth via snowballing effects on other marine organisms. One solution would be to boost coral resilience (i.e., increase stress tolerance and facilitate recovery after disturbances) by “human-assisted evolution” (van Oppen et al., 2015). In essence, one would select the most desirable phenotypes after stress exposure of natural stocks to induce preconditioning and transgenerational acclimation, or by selective breeding with standing or new genetic material (e.g., via hybridization with other populations or species). Although frequently discussed, these approaches remain largely theoretical. For example, genomic data and biophysical models indicate that gene flow from lower to higher latitude coral populations can offer beneficial heat-tolerant alleles as the climate warms (Matz et al., 2018). However, empirical studies are emerging, such as recent work exploiting assisted gene flow (using cryopreserved sperm) to enhance genetic diversity in isolated populations of coral threatened with extinction (Hagedorn et al., 2021).

The concept of preparing populations for climate change through assisted gene flow (Aitken & Whitlock, 2013) is receiving increased attention (Grummer et al., 2022). The idea is simple. When climate change outpaces the rate at which populations can adapt, phenotypes will become increasingly maladapted, resulting in population declines and ultimately, extinction. One reason why populations are incapable of adapting is insufficient genetic variation in potentially adaptive traits, especially in genetically isolated populations. Assisted migration replacing natural gene flow can thus be a powerful conservation tool, particularly for populations that are experiencing both rapid climate change and habitat fragmentation (Grummer et al., 2022).

Similar ideas have been put forward for overharvesting. Exploitation of fish populations is inducing rapid evolutionary responses in fish life history traits. For example, fisheries that target large individuals select for early maturation at smaller sizes, leading to reduced fecundity and thus also reduced fisheries yields (Heino et al., 2015), as observed in Atlantic cod (Olsen et al., 2004) and silverside (Conover & Munch, 2002). Management tools that preserve natural genetic variation include (a) reducing the intensity of the harvest, (b) limiting minimum and maximum body sizes culled in order to maintain a range of body sizes in the population; and (c) shifting the seasonal timing of fishing to ensure that larger fish can reproduce before they are captured (Kuparinen & Merilä, 2007). Olson et al. (2009) have suggested a more direct genetic intervention, by releasing fast-growing domesticated fish strains to interbreed with wild fish, thereby driving changes that are maladaptive in wild local populations (where rapid growth and large body size are selected against) but which would reverse some of the rapidly evolved demographic impacts of overharvesting. A Darwinian approach to fisheries management has been advocated (Dunlop et al.,

2009), but it is difficult to change the policies of a multibillion dollar business.

Another common theme for evolutionarily-informed management is pollution. For example, ectoparasitic salmon lice present a major challenge to aquaculture of Atlantic salmon (Coates et al., 2021). The overuse of chemicals for louse control has led to increased resistance of lice to common chemotherapeutic agents. One strategy to manage the evolution of resistance is by establishing wild refugia where fish are left untreated to maintain susceptibility to louse-control chemicals. Immigration of non-resistant genotypes of parasites from these wild hosts dilutes the frequency of parasite resistance in farmed fish. Gene flow in the other direction (of chemical-resistant parasite genotypes to wild fish) could weaken any advantages to this system, but these resistant genotypes may have low viability in wild salmon and hence may soon be lost (Kreitzman et al., 2018; McEwan et al., 2015).

In summary, the idea of exploiting evolutionary insights for conservation is widespread, but there are few concrete examples of the approach being put into practice. One major obstacle, as we have noted above, is a lack of detailed knowledge about rapid evolution in ecological systems under management. Some of the best examples of rapid evolutionary change come from studies of biological invasions, especially in cases where the invader has evolved in an ecosystem very different from that it is currently colonizing (Sax et al., 2007; Westley, 2011). Such an invader may be under intense selection to adapt to abiotic aspects of the invaded range, as well as having to deal with a novel suite of prey, predators, and pathogens (Carroll et al., 2005; Shine, 2012; Stuart et al., 2014). If co-evolved predators, prey, and pathogens are absent from the invaded range (“enemy release”), and their place is taken by organisms from different phylogenetic lineages, the phenotypic traits that enhance fitness in the invader’s interspecific interactions may differ dramatically between the native range and the invaded range. Likewise, intraspecific interactions may be modified by an invasion. High abundances typify some stages of colonization, increasing intraspecific competition within invasive populations (Brown & Shine, 2019; DeVore et al., 2021a). However, changes in optimal trait values are not the only driver of changes wrought by invasion. For example, prolonged range expansion can generate more rapid dispersal due to non-adaptive spatial sorting of traits (Shine et al., 2011).

Although conservation biologists are increasingly aware of the potential for rapid evolution, less attention has been paid to the management implications of that dynamic situation (Ashley et al., 2003). The most obvious consequence of rapid adaptation of an invader to novel challenges will be to facilitate population growth of the pest, rendering control in its new environment more difficult. However, rapid adaptation of native taxa to withstand the invader’s impacts may suggest ways to amplify that resistance; for example, the invasion of a highly toxic prey species may impose intense selection on predators to stop eating that prey type, suggesting that managers could buffer impacts of the invader by translocating predators with innate avoidance of that dangerous new prey type (Kelly & Phillips, 2019). An additional possibility is the focus of the current paper: the idea that evolutionary shifts in an invader may create vulnerabilities not present in the native range.

Few biological invasions have been studied in enough detail to quantify evolutionary shifts in traits relevant to

management. One exception involves the introduction of cane toads (*Rhinella marina*) to northeastern Australia in 1935, and the toad's subsequent spread westward through the Australian tropics (Shine, 2018). Research has documented rapid changes in the morphology, physiology, behavior, life history, and ecology of cane toads across their invasion trajectory (Gruber et al., 2017a; Hudson et al., 2016, 2018), and concurrent changes in morphology, physiology, and behavior of native wildlife taxa (predators) imperiled by the spread of the highly toxic anuran (Phillips & Shine, 2004, 2006). This provides an ideal system in which to explore the possibility that rapidly evolved traits may provide novel management tools.

## Rapid evolution in cane toads, their pathogens, and their predators

### Study species

Cane toads (*Rhinella marina*; formerly *Bufo marinus*) are among the largest bufonid anurans, with adults sometimes exceeding 1 kg in mass (Shine 2018; Figure 1). Adult toads are generalist predators that prey primarily on terrestrial and flying insects, and lay large clutches (to > 20,000 eggs) in non-flowing waterbodies. Within a day or two, the eggs hatch into small (0.1 g) tadpoles that develop rapidly and metamorphose into tiny metamorphs. The larval phase can be completed in a few weeks in hot nutrient-rich conditions, but is prolonged by lower temperatures or restricted food supply (Crossland & Shine, 2012; Crossland et al., 2012).

### Translocation history

The cane toad is native to rainforest and associated habitats in northeastern South America (Acevedo et al., 2016). The species was intentionally translocated from French Guiana to Puerto Rico and other Caribbean islands in the 1800s to consume insect pests in sugar-cane plantations, and 150 Puerto Rican toads were taken to Hawai'i in 1932 for the same purpose (Selechnik et al., 2017). Three years later 101 progeny of those toads were taken to northeastern Australia (Shine et al., 2020). The captive toads soon bred, and thousands of their offspring were released in sugar-cane plantations in coastal Queensland. Geographic expansion was slow initially,



**Figure 1.** The cane toad (*Rhinella marina*) is native to South America but was introduced to many countries around the world in a futile attempt to control insect pests in sugar-cane plantations. Photograph by Matt Greenlees, used with permission.

but invasion rates accelerated as the toads moved westwards through tropical Australia (Urban et al., 2008). Southward spread along the east coast has been slower (Macgregor et al., 2021).

### Ecological impact in the invasive range

Like most bufonids, cane toads produce toxic defensive chemicals in parotoid (shoulder) glands as well as in smaller glands distributed over the body surface (Hayes et al., 2009). Anurophagous predators with an evolutionary history of sympatry with bufonids have evolved genetic changes that massively decrease physiological vulnerability to bufadienolide toxins, but predators lacking that exposure history tend to be highly susceptible (Ujvari et al., 2013). In Australia, the spread of cane toads has caused widespread population declines of endemic predators such as northern quolls (*Dasyurus hallucatus*), freshwater crocodiles (*Crocodylus johnstoni*), yellow-spotted monitors (*Varanus panoptes*), and large elapid snakes (e.g., *Pseudechis* spp.) (Shine, 2010).

The types and amounts of bufadienolide toxins change ontogenetically within the toad's life cycle. Eggs contain diverse and abundant toxins, that protect against predator attack (Hayes et al., 2009). Fishes and turtles that consume toad eggs often die, perhaps because toxins beneath the egg's jelly-coat are undetectable at the time of ingestion (Somaweera et al., 2011). In contrast, tadpoles contain few toxins; fishes and birds soon learn not to eat toad larvae but rarely die (Caller & Brown, 2013; Nelson et al., 2011). Post-metamorphosis, the toad begins to manufacture its own toxins; consuming a large (adult) cane toad is far more likely to be fatal to a predator than consuming a small metamorph (Crossland & Shine, 2010; Cabrera-Guzmán et al., 2013).

### Rapid evolution in cane toads

Geographic comparisons reveal strong divergences in phenotypic traits between cane toads in the native range (French Guiana), in Hawai'i, and in Australia. The most dramatic changes occur across Australia, and involve the modification of traits (e.g., leg lengths, body shapes, locomotor endurance, boldness) that enhance rates of dispersal (Gruber et al., 2017a; Hudson et al., 2016, 2018). Those higher dispersal rates trade off with investment into immunocompetence (Llewellyn et al., 2012) and reproduction (Hudson et al., 2015; Kelehear & Shine, 2020). Common-garden breeding experiments reveal that many of the distinctive phenotypic traits of local toad populations are heritable (i.e., have a genetic underpinning: Brown et al., 2014; Shine, 2012) although phenotypic plasticity is important also (Hudson et al., 2018; Phillips et al., 2010a).

One striking evolutionary shift involves cannibalism. Toad tadpoles from all Australian populations tested to date are highly cannibalistic on younger conspecifics. Chemicals (toxins) released as eggs hatch powerfully attract older toad tadpoles and stimulate vigorous feeding behavior, often resulting in eradication of a newly laid clutch of eggs (DeVore et al., 2021a). In contrast, toad tadpoles from French Guiana and Hawai'i are not attracted to those chemicals and rarely consume conspecific eggs (DeVore et al., 2021a). The cannibalism of Australian toad tadpoles is species-specific, with the eggs of native anurans ignored (Crossland et al., 2022). The rapid evolution of this behavior, and of adaptive acceleration of development by vulnerable hatchlings that detect chemical cues from cannibalistic conspecifics, has been attributed to

more intense intraspecific competition in Australia than in the native range (DeVore et al., 2021a).

### Rapid evolution in the pathogens of cane toads

Many parasites and pathogens that infect cane toads in the native range were left behind during the toad's translocation (DeVore et al., 2020; Selechnik et al., 2017). However, many toads in Australia are infected with nematode lungworms (*Rhabdias pseudosphaerocephala*) that accompanied the founding individuals (Dubey & Shine, 2008). These lungworms can reduce rates of feeding, growth, and survival in cane toads (Finnerty et al., 2018; Kelehear et al., 2011) and are absent from the invasion front because infected toads exhibit lower dispersal rates (Phillips et al., 2010b). Lungworms from populations closer to the invasion front have evolved larger egg size and accelerated maturation, relative to lungworms in long-colonized areas (Kelehear et al., 2012; Schlippe Justicia et al., 2022). Cross-infection experiments reveal that the ability of a toad to resist infection, and the ability of lungworm larvae to infect a new host, differ considerably among geographic host-parasite combinations (Mayer et al., 2021).

### Rapid evolution in predators affected by cane toads

Comparisons within Australia reveal marked divergences in “toad-smart” traits between populations of vulnerable native predators that are sympatric *versus* allopatric with toads, or that have been exposed to toads for differing periods of time (Caller & Brown, 2013; Phillips & Shine 2006; Shine, 2010). Predators without exposure to toads often attempt to consume the toxic anuran (and are fatally poisoned as a result) whereas predators from toad-infested areas typically delete toads from the diet (Pettit et al., 2020). That shift may be largely driven by plasticity, with Conditioned Taste Aversion documented in fishes, anurans, lizards, crocodiles, and marsupials (Nelson et al., 2011; O'Donnell et al., 2010; Price-Rees et al., 2013; Ward-Fear et al., 2016; Webb et al., 2011). Nonetheless, these shifts may be reinforced by heritable changes (i.e., genetic assimilation), with taste-aversion present in toad-exposed taxa unable to learn aversion (e.g., elapid snakes; Phillips & Shine, 2006) and innate toad avoidance in young quolls (Kelly & Phillips, 2019). Physiological resistance to toxins also may evolve (Phillips & Shine, 2006) but no Australian predators are known to have rapidly acquired the genetic changes that characterize long-term co-evolved anurophagous predators (Ujvari et al., 2013).

## Exploiting rapidly evolved changes for conservation

### Rapidly evolved changes in rate of dispersal

#### Predicting rates of spread

Managers can plan most effectively if they can predict the rate at which a range-expanding species will spread. The obvious null hypothesis is to expect future rates of spread to resemble those seen earlier in the invasion, but an extensive body of recent literature indicates that accelerated rates of range expansion through time are more likely. Accelerating rates may evolve if invasion-front individuals have higher fitness, due to better access to resources because of low densities of conspecifics (as is true in cane toads: Brown et al., 2013). More generally, the non-adaptive process of spatial sorting will cause invasional acceleration as dispersal-enhancing

alleles accumulate at an increasingly fast-moving range edge (Shine et al., 2011). This mechanism entails evolution based on differential rates of dispersal rather than differential fitness (unlike natural selection), and rests upon repeated episodes of positive assortative mating between the fastest dispersers at the range edge (Shine et al., 2011). Some progeny from those matings will inherit alleles for “fast dispersal” traits (morphology, physiology, behavior) from both parents, amplifying dispersal rate.

The generality of invasional acceleration is supported by mathematical modelling (e.g., Chan et al., 2015; Phillips & Perkins, 2019), laboratory experiments with organisms such as bacteria and *Tribolium* beetles (Weiss-Lehman et al., 2017), and by field-based evidence that dispersal-enhancing phenotypic traits accumulate at invasion fronts (e.g., lighter seeds in plants, larger feet in rodents: Forsman et al., 2011; Huang et al., 2015) and increased rates of range expansion through time (e.g., Narimanov et al., 2022). In cane toads, accelerated invasion is shown by mapping across Australia (Urban et al., 2008) as well as by radio-tracking studies of cane toads (Shine et al., 2021).

Knowing that invasions accelerate because of rapid evolution can facilitate the planning of efforts at eradication or control, or fine-tuning the times at which imperiled native taxa are brought into captivity to set up “insurance populations” (e.g., Morrison et al., 2020).

### Reducing rates of spread and/or the extent of invaded area

High rates of range expansion by the invader are a management problem for two reasons. First, they reduce the time available to plan and implement curtailment strategies. Second, a fast-dispersing invader may be able to cross barriers of “unsuitable” habitat in brief windows when conditions are amenable to invader persistence. For example, an invasive taxon reliant on warm conditions may penetrate into seasonally cold regions during summer (McCann et al., 2017); and a faster-dispersing invader can occupy a larger area (and reach suitable habitat on the other side) before being eradicated by falling temperatures in autumn.

In the case of cane toads in tropical Australia, aridity is the major abiotic barrier to range expansion. Unlike native anurans that are inactive during the dry season, cane toads remain active year-round and need to rehydrate on an almost-daily basis (Tingley & Shine, 2011; Webb et al., 2014). Thus, a wide barrier of dry habitat may be unsurvivable by cane toads except during the relatively brief wet season. Such a barrier may prevent further expansion of the toad invasion front if the distance to be covered exceeds the distance that a toad can disperse during the wet season (Phillips et al., 2016). That annual dispersal distance is around 10–15 km for toads from long-colonized areas of Queensland, but over 60 km for toads at the invasion front in Western Australia (Urban et al., 2008). Thus, the evolved acceleration of dispersal rate may enable toads to extend their range into areas that would be inaccessible otherwise.

We might be able to restrict the toads' expansion across arid barriers with a “genetic backburn” (Phillips et al., 2016). That is, we could translocate toads from long-colonized (low-dispersal-rate) populations in eastern Australia, to a site in advance of the current invasion front. When the colonizers at the invasion front arrived, they would breed with the translocated animals to produce offspring that exhibit some intermediate level of dispersal rate—and thus, are less capable

of crossing an arid barrier. In essence, we could break apart the “high-dispersal-rate” syndrome. Modelling suggests that this strategy might work, especially if combined with elimination of artificial water-sources along the dry corridor (Phillips et al., 2016).

The feasibility of a genetic backburn is enhanced by other rapidly evolved traits in invasive toads. Individuals at the front have experienced generations of natural selection under conditions of low conspecific densities. By definition, the abundance of toads is zero in advance of the front, and close to zero immediately behind it. Life-history theory predicts that the resultant reduction in intraspecific competition should favor the evolution of “*r*-selected” life-history traits such as small egg size and low competitive ability, if such changes enable higher fecundity or come at minimal cost to larval viability. Consistent with those predictions, egg size is reduced at the invasion front (J. DeVore, S. Ducatez, M. Crossland, and R. Shine, unpublished data) and tadpoles from invasion-front populations are competitively inferior to tadpoles from populations in long-colonized areas (Clarke, 2019; Ducatez et al., 2016).

A second set of rapidly evolved traits is relevant also. Toads from the invasion front trade off energy expenditure for dispersal with investment into other functions, including reproduction. Male toads from invasion-front populations have smaller testes than do males from long-colonized areas (Friesen & Shine, 2019). Under standardized conditions, female toads from the invasion front produce fewer clutches of eggs than do females from long-colonized areas (means of 0.25 vs. 1.0 clutch per year: Hudson et al., 2015).

In combination, these rapidly evolved traits of invasion-front toads (low competitive ability, low reproductive rate) should enable translocated toads from long-colonized areas to dominate the cohort of metamorphs emerging from mixed breeding sites. That competitive advantage should further enhance the impact of a genetic backburn, rendering the progeny even less capable of crossing barriers of unsuitable habitat.

### Tailoring management approaches to exploit local adaptation to abiotic challenges

The westward invasion of toads across tropical Australia has exposed them to climatic conditions much hotter and seasonally drier than are encountered in the species’ native range (Kosmala et al., 2020a; Tingley et al., 2014). In response, toads have evolved traits such as an increased locomotor ability when hot or desiccated (Kosmala et al., 2018) and an ability to utilize evaporative cooling (Kosmala et al., 2020b). However, the primary shift is an ecological one. Because the wider landscape is lethally dry for a toad, arid-zone populations are restricted to the immediate vicinity of waterbodies (Brusch et al., 2019; Tingley & Shine, 2011; Webb et al., 2014). Counter-intuitively, then, selection has favored adaptations of hydric balance usually expected in anurans from mesic not arid habitats (Kosmala et al., 2020a, c). For example, toads from arid habitats in Western Australia have unusually thin skin, with high rates of water loss under dry conditions (Kosmala et al., 2020c).

Managers can exploit the rapid evolution of mesic-habitat adaptations in arid-zone toads by focusing control efforts on removing access to water. Fencing an isolated waterbody in an arid site to prevent toads from returning to the pond kills most of the resident toads from nearby areas within one

to two nights (Tingley et al., 2017). That management tactic would be less effective if the toads retained the ancestral condition of thicker skin and lower rates of water loss (Kosmala et al., 2020a): rapid evolution has created a habitat dependency that managers can exploit. Control techniques such as hand-collecting and trapping toads may be methods of choice in mesic areas (Greenlees et al., 2020; Shine et al., 2018) but exclusion fencing around waterbodies may be more effective in arid regions.

### Tailoring management to exploit local adaptation to biotic challenges

#### Exploiting evolved shifts in social behavior of toads

In laboratory trials, invasion-front toads exhibit higher levels of aggregation (Gruber et al., 2017b), possibly reflecting the lower densities of conspecifics at the front (reducing mating opportunities) and harsh environmental conditions (such that a conspecific’s presence predicts habitat suitability). Attraction to conspecifics at the invasion front might facilitate trapping using cues that cane toads use to predict the presence of conspecific toads. Likewise, Yasumiba et al. (2016) documented rapidly evolved shifts in the acoustic characteristics of the calls of male cane toads; and synthetic calls that amplify the attractive components of natural calls attract toads into traps (Muller & Schwarzkopf, 2017). In short, managers could fine-tune control methods relative to the behavioral attributes of toads in local populations.

#### Exploiting host-parasite arms races

Toads near the invasion front invest less in immune defense (Llewellyn et al., 2012) and modify the form of that investment (Brown et al., 2015). Also, at least one major parasite (a nematode lungworm brought with the toad from South America) has evolved changes in traits such as egg size and time to maturity (Kelehear et al., 2012). As a result, toads from different regions of Australia are differentially vulnerable to lungworms from different regions. For example, lungworms from Western Australia are more capable of infecting Northern Territory toads than are Northern Territory strains (Mayer et al., 2021). The geographic variation in lungworm virulence and toad resistance suggests that we might be able to suppress local toad populations by introducing strains of lungworms from other regions; that is, strains more capable of breaching host defenses than are locally occurring strains.

#### Exploiting rapidly evolved mechanisms for intraspecific competition

##### Intensifying intraspecific competition by concentrating spawning in space or time

Cane toads in Australia engage in intense intraspecific rivalry via cannibalism and competition for food (in both aquatic and terrestrial phases: Crossland et al., 2022; Pizzatto & Shine, 2008), and chemical suppression (aquatic phase only). Although we lack detailed information from the native range, low population densities and sympatric bufonids may render intraspecific interactions less important than interspecific competition. Two important components of larval competition—cannibalism and suppression—are widespread in Australia but not in the native range (DeVore et al., 2021a).

We could intensify intraspecific competition in invasive populations by concentrating spawning in time and space. If some potential spawning sites were rendered unavailable

(perhaps by fencing) or unattractive (perhaps by adding cane toad tadpoles: McCann et al., 2020, and see below), we could concentrate toad breeding in the remaining sites. Such a concentration would exacerbate intraspecific competition, reducing recruitment rates from the remaining waterbodies.

### Using intraspecific cues to attract and cull larvae

Unlike conspecifics in the native range, larval cane toads in Australia are strongly attracted to bufadienolide toxins (Crossland et al., 2012). Funnel traps baited with such toxins can eradicate cane toad tadpoles from natural waterbodies (Crossland et al., 2012; Figure 2). The baits can be taken from the parotoid glands of adult cane toads, and the researchers who developed this method have licensed the technology to a nonprofit group who provide baits to the general public (see <https://watergum.org/greatcanetoadbust/>). Uptake has been swift, with “toad-busting” groups using the method to remove millions of cane toad tadpoles from waterbodies across tropical Australia (see Shine, 2018).

### Using toads to control their own populations

Tadpole trapping is time-intensive and labor-intensive, and must be repeated frequently given the brief duration of larval life in the species (<4 weeks in hot, nutrient-rich conditions: e.g., McCann et al., 2020). To overcome those logistical challenges, one of us (R.S.) has proposed an alternative way to exploit the intense intraspecific competition that has evolved in invasive cane toads within Australia.

The new approach builds on the evidence that cane toad larvae pose little threat to native predators (because of low toxin levels: see above) but powerfully suppress younger conspecifics through cannibalism, competition for food, and chemical suppression. The latter mechanism involves induction of accelerated development in toad eggs that detect chemical cues from nearby cannibalistic toad tadpoles; an egg detecting those cues develops more rapidly through the vulnerable hatchling stage (DeVore et al., 2021a, b). Like cannibalism, this developmental acceleration is not seen in the toad’s native range (DeVore et al., 2021a). Especially in small eggs (typical of the invasion-front population, because of *r*-selection), the acceleration of development results in

severe malformations (Clarke et al., 2015). The combined effects of cannibalism, competition, and chemical suppression largely eliminate eggs laid into a pond that already contains toad tadpoles (>95% mortality: Clarke et al., 2015; DeVore et al., 2021b).

In short, larval cane toads pose little risk to native species but massively curtail intraspecific recruitment. If we prevented cane toad tadpoles from metamorphosing, to retain a larval population long term, they would destroy any toad eggs laid into that pond (Figure 3). In other anurans such as *Xenopus laevis*, mutant non-metamorphosing tadpoles can live for years (Rot-Nikcevic et al., 2004). The physiological and genetic pathways that drive anuran metamorphosis are well studied, facilitating the application of CRISPR-Cas9 methods to knock out genes required for that metamorphosis. This approach uses cane toads to control their own populations, by intensifying intraspecific competition.

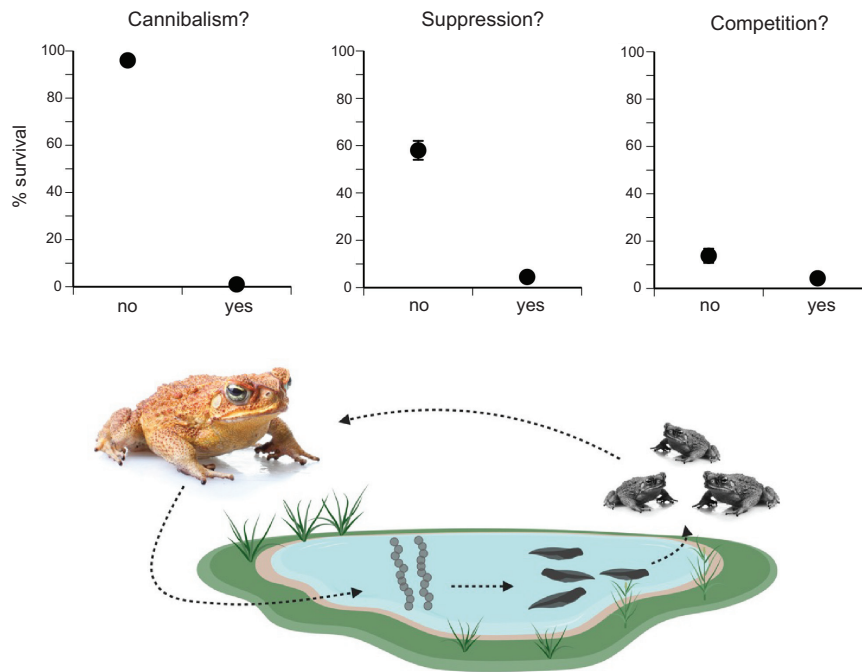
Although the technical challenges are considerable, this idea identifies an exciting new approach for pest control. In a target species with a multiphasic life history, simple genetic modifications (gene knockouts) might disrupt the life cycle in a way that devastates pests but has little impact on vulnerable native species. Because the CRISPR component of the work involves only gene knockout rather than transfer of genetic material, it avoids many of the regulatory impediments to release of GMOs—and also overcomes many arguments from community groups against GMO approaches. Critically for the subject of this paper, that opportunity has arisen only because cane toads in Australia have rapidly evolved larval cannibalism in response to intense intraspecific competition.

### Fine-tuning control methods to maintain their effectiveness

All control methods impose selection for traits that enable individuals to escape the cull. For example, culling adults imposes selection for early maturation; traps impose selection for trap-avoidance behaviors. A trait that has evolved only recently may be more likely to reverse rapidly under such selection imposed by management. In the case of cane toads, for example, traps that use artificial lights to attract insects and thus toads would select against “bold” toads that are the



**Figure 2.** The parotoid (shoulder) glands of adult cane toads (*Rhinella marina*) contain copious amounts of bufadienolide toxins that can be squeezed out of the glands by manual pressure (left panel). The exudate is a powerful attractant to conspecific tadpoles, and hence can be used as bait in funnel traps to selectively eradicate toad tadpoles from waterbodies (right panel). Photographs by Greg Brown, used with permission.



**Figure 3.** Life cycle of the cane toad (*Rhinella marina*), to illustrate the idea that blocking metamorphosis could eliminate recruitment to the terrestrial phase, while providing a cohort of long-lived larvae that prevent toad-breeding in that pond through cannibalism of eggs, chemical suppression of hatchlings, and competition with post-hatching larvae. The upper panels show the magnitude of reduction in larval survival due to the presence of older toad tadpoles as a consequence of cannibalism (DeVore et al., 2021a), chemical suppression (Clarke et al., 2016), and competition for food (Crossland et al., 2011). The lower panel depicts the life cycle of the cane toad; blocking the transition from larvae to metamorphs could eliminate recruitment from future as well as present cohorts.

first to approach such a light (Gonzalez-Bernal et al., 2014). Likewise, tadpole trapping using toxin as bait would select for larvae that are not attracted to that cue.

The ideal management approach renders such evolutionary responses less likely or less effective. For example, if we used CRISPR to block metamorphosis, we would ideally knock out multiple genes in thyroxin pathways, so that no single mutation could restore effective function. Similarly, the non-metamorphosing tadpole approach rests on three mechanisms of control of younger tadpoles by older ones: cannibalism, chemical suppression, and competition. Mutations that rendered a clutch less vulnerable to one of those processes might not increase fitness, if the entire clutch was strongly affected (ideally, eliminated) by the other mechanisms. In short, managers can use evolutionary thinking to predict and block pathways by which the targeted pest species might adapt to escape from control.

### Using rapidly evolved “toad-smart” traits to buffer vulnerable predators

#### Genetic translocation

The spread of toxic cane toads across tropical Australia has caused population declines of large-bodied predators, imposing intense selection for traits that enable survival in a toad-infested region. Many toad-sympatric predators delete toads from their diet (e.g., Pettit et al., 2020) via Conditioned Taste Aversion (Shine, 2010). Also, selection has favored alleles that discourage toad consumption by more rapid learning or innate aversion in at least three species, one snake (Phillips & Shine, 2006), one fish (Caller & Brown, 2013) and one marsupial (O'Donnell et al., 2010). Innate toad avoidance occurs in the offspring of quolls from toad-exposed populations (Kelly & Phillips, 2019).

Translocating predators with “toad-smart” traits such as behavioral aversion or enhanced toxin resistance might enable newly exposed predator populations to recover more quickly (Kelly & Phillips, 2019). Translocation of toad-smart traits before the arrival of toads might even buffer the initial population decline.

#### “Insurance populations”

For critically endangered taxa, one option of last resort is to bring survivors into captivity to prevent extinction and to build up numbers for subsequent release. The rapid evolution of toad-smart traits suggests that such “insurance populations” ideally should be founded by survivors of the toad invasion rather than by individuals collected from toad-free areas. Collection from already-affected areas imposes logistical challenges (the predators are already rare) and ethical issues (removing the last individuals of that species in the landscape) but the founder individuals likely have genetic traits that facilitate coexistence with the threatening process.

#### Artificial selection for toad-smart traits

Rapid evolutionary change can be a problem as well as an opportunity for managers. For example, an “insurance population” held in captivity for several generations may lose alleles underpinning important responses in the wild. Quolls translocated to a predator-free island lost antipredator responses within 13 generations, reducing their survival rates post-release (Jolly & Phillips, 2021; Jolly et al., 2018). One solution may be to impose relatively weak selection in captive populations, for example by introducing small numbers of small toads, or a few predators (Moseby et al., 2016).

## Concluding remarks

Based largely on examples from the cane toad invasion of Australia, we suggest that an understanding of rapid evolutionary change can provide opportunities for novel conservation interventions. Nonetheless, such opportunities have rarely been exploited. Even in the case of the toad system, only a single method—the use of toxin-based baits to cull larvae of the invasive toad—has progressed to widespread use. The other ideas we review above are simply ideas, yet to be refined and applied.

One of the main impediments to exploiting rapid-evolved vulnerabilities to control invasive species is a lack of fundamental research on such systems. It required decades of research to understand the cane toad invasion in enough detail to detect such vulnerabilities, and most of the exploitable opportunities (such as larval attraction to toxins, and suppressive effects of chemical exposure to older tadpoles) arose from fundamental rather than applied research (see Shine, 2018). Funding for invasive-species control typically focuses on short-term activities that directly reduce invader abundance, rather than strategic long-term research to understand the dynamics of invader-native interactions. The example of the cane toad suggests that investment into fundamental science can ultimately be more cost-effective than efforts to deploy conventional weapons against the invader.

In the example of the cane toad, long-term research also identified Conditioned Taste Aversion (CTA) as the mechanism that buffers toad impact on vulnerable native predators (Shine, 2010). In turn, that discovery led to field trials that imposed CTA by exposing predators to small (CTA-inducing) rather than large (lethally toxic toads) in advance of toad invasion, in order to reduce predator mortality (e.g., Ward-Fear et al., 2016). The success of those trials then stimulated landscape-scale deployment of aversion-inducing small toads in advance of the front, leading to successful buffering of toad impacts (Ward-Fear et al., unpublished data; see [www.canetoadcoalition.com](http://www.canetoadcoalition.com)). Although that management initiative does not build on rapid evolution, it further emphasizes the potential rewards of understanding a biological invasion in order to mitigate its impacts.

## Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## Author contributions

R.S. and S.B. jointly developed the idea for this paper and contributed equally to conceptualization and writing. R.S. is responsible for the novel control approach using non-metamorphosing tadpoles.

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