THE QUARTERLY REVIEW
of BIOLOGY

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NEW WEAPONS IN THE TOAD TOOLKIT: A REVIEW OF METHODS TO CONTROL AND MITIGATE THE BIODIVERSITY IMPACTS OF INVASIVE CANE TOADS (*RHINELLA MARINA*)

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ABSTRACT

Our best hope of developing innovative methods to combat invasive species is likely to come from the study of high-profile invaders that have attracted intensive research not only into control, but also basic biology. Here we illustrate that point by reviewing current thinking about novel ways to control one of the world's most well-studied invasions: that of the cane toad in Australia. Recently developed methods for population suppression include more effective traps based on the toad's acoustic and pheromonal biology. New tools for containing spread include surveillance technologies (e.g., eDNA sampling and automated call detectors), as well as landscape-level barriers that exploit the toad's vulnerability to desiccation—a strategy that could be significantly enhanced through the introduction of sedentary, range-core genotypes ahead of the invasion front. New methods to reduce the ecological impacts of toads include conditioned taste aversion in free-ranging predators, gene banking, and targeted gene flow. Lastly, recent advances in gene editing and gene drive technology hold the promise of modifying toad phenotypes in ways that may facilitate control or buffer impact. Synergies between these approaches hold great promise for novel and more effective means to combat the toad invasion and its consequent impacts on biodiversity.

INTRODUCTION

PREVENTING nonnative species from reaching new areas via quarantine is typically the most effective strategy to combat biological invasions, although eradicating newly established populations can be feasible with intensive effort (Simberloff 2003). Once an invader begins to spread, however, eradication success declines markedly, and substantial population reductions may be difficult to achieve. Effective control methods may take decades to develop and, with a few exceptions (Shanmuganathan et al. 2010), are unlikely to succeed (Saunders et al. 2010). Nonetheless, the potentially devastating ecological impacts of invasive species (Kraus 2015; Bellard et al. 2016) mean that we urgently need to develop and apply new control and mitigation methods. Our best hope of success may come with high-profile pest species that have attracted intensive research not only into control, but also basic biology (Simberloff 2003). In this paper, we illustrate the importance of understanding the fundamental biology of invaders by reviewing current ideas (most of them developed very recently) on ways to control and reduce the ecological impact of the world's most intensively studied amphibian invasion: the deliberate release and subsequent spread of the cane toad (*Rhinella marina*, formerly *Bufo marinus*) in Australia (Figure 1).

Native to Latin America, these exceptionally large (up to more than 1 kg) and highly toxic anurans were released along the northeastern coast of Australia in 1935 in a futile attempt to control insect pests in commercial sugarcane plantations (Turvey 2013). The toads have since spread at an increasing rate across more than 1.2 million km² of tropical and subtropical Australia (Urban et al. 2008), and have not yet filled their fundamental niche, either in Australia (Kearney et al. 2008) or globally (Tingley et al. 2014). Over the course of their Australian invasion, cane toads have had devastating impacts on native biodiversity (Shine 2010). In particular, large anuran-eating predators (such as

Figure 1. A Male Cane Toad From the Northern Territory, Australia
Photo by Ben Phillips. See the online edition for a color version of this figure.

marsupial quolls, freshwater crocodiles, varanid and scincid lizards, and elapid snakes) are fatally poisoned when they ingest the toxic invaders. In many areas, toad invasion has caused precipitous declines in predator abundance (Letnic et al. 2008; Doody et al. 2009, 2014; Shine 2010; Jolly et al. 2015; Fukuda et al. 2016). These declines have, in turn, had flow-on effects to other species via trophic cascades (Brown et al. 2011b; Doody et al. 2013, 2015).

Despite considerable financial investment (e.g., more than \$20 million from 1986–2009; Commonwealth of Australia 2011), the spread of cane toads across Australia has continued unabated. Initial control efforts in the wet-dry tropics of northern Australia focused on manually collecting adult toads and have been largely unsuccessful. The feasibility of more sophisticated genetic-based biocontrol methods, such as virally vectored autoimmunity (Robinson et al. 2006; Pallis-

ter et al. 2008, 2011), other viral-based, gene deletion and RNAi-based control strategies (Shanmuganathan et al. 2010), and sex-biasing mechanisms (Koopman 2006; Mahony and Clulow 2006) have been considered and occasionally (in the case of virally vectored strategies) extensively investigated (Hyatt et al. 2008). Their likelihood and context of success for managing toads have also been analyzed (McCallum 2006; Thresher and Bax 2006), but technical obstacles (and concerns about unintended consequences) prevented their successful development and implementation (Hyatt et al. 2008; Shannon and Bayliss 2008).

Consequently, when the state of efforts to control cane toads was last reviewed by the Australian Federal Government in 2011, it seemed likely that the species would fully colonize its potential range before any suitable technology for broad-scale control became available (Commonwealth of Australia

2011). In its threat abatement plan, the Australian Commonwealth therefore decided to move away from broad-scale control and eradication, and focus instead on the protection of key biodiversity assets, such as offshore islands and priority native species and ecological communities (Commonwealth of Australia 2011). Suitable approaches for achieving that aim were largely unavailable at the time, but a recent (June 2016) workshop to review the threat abatement plan revealed substantial advances in our understanding of chemical ecology, genetic technologies, mechanisms of toad impact, and fundamental cane toad biology. The participants of that workshop identified exciting opportunities to capitalize on these recent advances to develop innovative management approaches for a variety of applications, including at and behind the invasion front, and on offshore islands (Table 1).

In this paper, we provide an overview of these recently proposed approaches and developments. We consider three main types of management goals: suppressing toad population sizes; containing toad spread; and mitigating toad impacts on biodiversity. For each goal, we summarize available management tools and provide, where available, evidence for their effectiveness. We also speculate on the effectiveness of each management tool in different climatic regions of the toad's Australian range, and highlight potential barriers to implementation. We conclude with potential integrated strategies that draw on multiple management approaches. Our examples are focused on the Australian cane toad invasion, but the approaches we present here will be applicable to other countries throughout the Caribbean Sea and Indian Ocean where cane toads have established non-native populations, as well as to amphibian invasions more generally (Kraus 2015; Measey et al. 2016). Additionally, the ideas generated from intensive research on cane toads may suggest novel approaches to control a wide variety of other invasive organisms.

Population Suppression

Most efforts at controlling cane toads have focused on reducing toad abundance; these range from direct manual collection and trapping of adults to chemical suppression of larval development.

manual removal

The earliest terrestrial stages of the cane toad are largely diurnal, and restricted to the margins of natal ponds during dry weather conditions, particularly in the wet-dry tropics (Child et al. 2008a,b; Pizzatto et al. 2008). Metamorph toads can be collected or killed in large numbers if located before dispersing into the surrounding habitat matrix. Community groups have sprayed chemicals to kill metamorph toads, but the adverse ecological impacts of this practice (e.g., on native ants) have led to it being banned by environmental-management authorities in some parts of the toad's Australian range (Kelehear et al. 2012). Chemicals that were commonly used for this purpose (e.g., Dettol™) also kill lungworm parasites that might otherwise impair toad viability (Kelehear et al. 2012).

Juvenile and adult toads are primarily nocturnal, and often prefer relatively open habitat at night when foraging (González-Bernal et al. 2015). Adult males typically gather close to waterbodies, whereas adult females are often dispersed through the surrounding habitat (González-Bernal et al. 2015). Thus, hand collecting can result in male-biased captures if removal is focused exclusively on waterbodies. Radiotelemetric monitoring suggests that on nights with unsuitable weather conditions, a high proportion of adult toads remain inactive and, hence, are unavailable to collectors (Schwarzkopf and Alford 1996, 2002). Nonetheless, hand collecting can remove a significant proportion of adult toads within an area (Somaweera and Shine 2012). The spatial and temporal impact of such activities differs among sites and seasons. Manual removal of adult toads has a minor and short-term impact on toad abundances in areas close to the invasion front in the tropics, where fast-dispersing toads from nearby areas soon replenish local densities (Somaweera and Shine 2012). However, manual collection has longer-term impacts during the tropical dry season (when toads are sedentary; Brown et al. 2011a) and in southern (cool climate) areas (M. J. Greenlees and R. Shine, unpublished data). Manual

TABLE 1

Management goals and tools for controlling cane toads in Australia

Management goal	Action	Life stage	Time frame	Barrier(s) to implementation
Population suppression	Manual removal	Postmetamorphic/tadpoles/eggs	Short-term	High dispersal and reproductive rates mean long-term reductions are difficult
	Fencing waterbodies	Postmetamorphic	Short-term	Fence maintenance; collateral impacts
	Traps for adults	Postmetamorphic	Short-term	High dispersal and reproductive rates mean long-term reductions are difficult; checking traps remotely
	Traps for tadpoles	Tadpoles	Short-term	High dispersal and reproductive rates mean long-term reductions are difficult
	Suppression pheromones	Tadpoles	Medium-term	High dispersal and reproductive rates mean long-term reductions are difficult
	Using native species to reduce toad numbers	Tadpoles	Short-term	Effects on nontarget species
	Using pathogens for toad control	Postmetamorphic/tadpoles	Medium-term	Effects on nontarget species; current lack of suitable agent
Containment	Environmental DNA sampling	Postmetamorphic/tadpoles/eggs	Short-term	Does not remove toads
	Automated call detection devices	Postmetamorphic	Short-term	Does not remove toads
	Pilbara Line (waterless barrier)	Postmetamorphic	Medium-term	Stakeholder willingness; design of leak-proof tank and trough systems
Impact mitigation	Genetic backburning	Postmetamorphic	Medium-term	Public acceptance
	Conditioned taste aversion	N/A	Short-term	Public acceptance
	Targeted gene flow	N/A	Short-term	Public acceptance
	Gene banking and assisted reproduction	N/A	Short-term to medium-term	Public perception that conservationists have "given up"
	Gene editing and genome engineering (reducing toad toxicity)*	Postmetamorphic	Medium-term	Proof of concept; public acceptance

Listed for each action is the targeted life stage (postmetamorphic, tadpole, or egg), the amount of time needed for implementation (short-term: now–2 years; medium-term: 2–5 years), and important barriers to implementation and/or success.

* Detoxified toad, sex change, or sex-specific mortality/infertility, inducible mortality or disease susceptibility.

collection has been used to locally eradicate an island population of cane toads off Bermuda (Wingate 2011), and appears to have contributed to the decline and disappearance of two separate, extralimital populations south of the main distributional range on the eastern Australian seaboard (in Port Macquarie: White 2007; and more recently in Sydney: M. J. Greenlees and R. Shine, unpublished data).

In summary, manual collection and removal of juvenile and adult cane toads can be useful in some places at some times, most notably to eradicate satellite populations. It also has potential as a conservation tool in restricted areas (such as islands) where the conservation benefit may be high (e.g., to prevent sympatry with particularly sensitive species). However, in order to be most effective, manual collection needs to be integrated with other approaches (e.g., to capture individuals over longer periods or to eliminate breeding). Manual collection of adult toads has garnered sustained enthusiasm by the public in various parts of Australia and requires little expertise or equipment and, thus, is well-suited to community engagement (as long as humane euthanasia protocols are in place; Shine et al. 2015). Most large-scale removal efforts have occurred at the northern and southern invasion fronts, but population reduction in long-established areas can also be important for rallying public support for toad control, as human population densities can be much higher in these regions.

Disadvantages of manual removal include: the risk that untrained community members will inadvertently collect and kill native frogs (Somaweera et al. 2010); and the risk that if employed in isolation, or without strategic consideration of spatial-temporal context, ineffectiveness of manual collecting will render the public cynical about the possibility of controlling toads and, thus, reluctant to adopt other methods (although support has been sustained in some regions where toads are long established, such as Townsville, Queensland).

FENCING WATERBODIES

The dominant land use in semiarid Australia is cattle grazing, and to provide water for cattle, pastoralists have created numerous artificial watering points (AWPs; James et al. 1999). These typically consist of earthen bore-fed dams, which gravity feed water to raised cattle troughs fitted with float valves. In areas devoid of natural water, earthen dams serve as dry season invasion hubs from which cane toads disperse during wet season rains (Letnic et al. 2015).

Cane toads cannot survive without frequent access to water, or at least damp substrates (Florance et al. 2011; Tingley and Shine 2011; Jessop et al. 2013; Letnic et al. 2014; Webb et al. 2014) and, thus, fencing dams during the dry season is a simple way to eradicate toads in arid and semiarid landscapes (Florance et al. 2011). Fences can be constructed with star pickets, fencing wire, and shade cloth (Figure 2A); a team of workers can erect a fence in approximately three hours. An effective toad fence consists of two wires, one 600 mm high, and one flush with the ground, to which a roll of shade cloth is attached with metal fencing clips. Fences should be constructed on the flat crest of the dam wall, and should include a soil-covered 400 mm flange of shade cloth extending outward to prevent toads from burrowing under the fence (Letnic et al. 2015). Fences not only exclude toads from water, but also facilitate the hand collection of toads trapped inside the fenced area.

Fences are particularly effective for eradicating toads in arid to semiarid environments if they are constructed during the late dry season when toads experience significant thermal and hydric stress (Schwarzkopf and Alford 1996; Jessop et al. 2013). In a replicated field experiment in the Victoria River district, local eradication of toads was achieved at fenced dams after seven days (Florance et al. 2011). Fences had no adverse effects on native wildlife; frog mortality was negligible, and birds, pythons, kangaroos, and dingoes easily traversed the fences (Florance et al. 2011). Despite no maintenance for a year, the fences remained functional. One year later, the fenced dams acted as ecological traps, attracting toads to a water source they could no longer access. Thus, fences not only eradicated toads, but prevented them from reestablishing in the area the following dry season (Letnic et al. 2015).
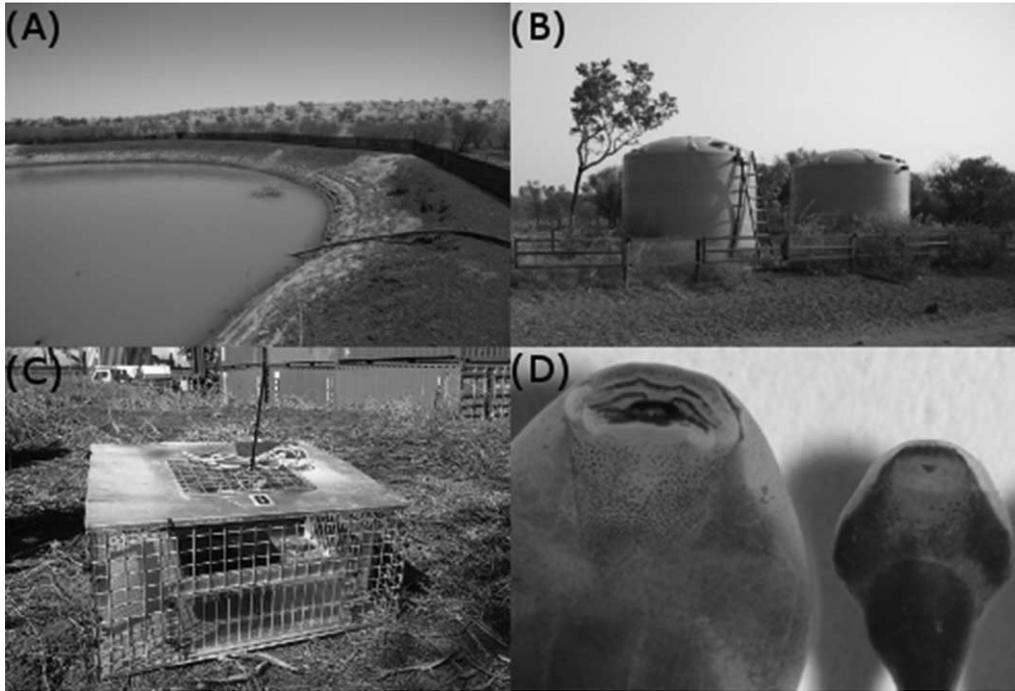
Figure 2. Examples of Approaches to Suppress Cane Toad Populations

(A) A cane toad exclusion fence erected around a farm dam in the Northern Territory, Australia. Photo by Jonathan Webb. (B) Tanks used to store water for cattle on a pastoral station in the Northern Territory. Such tanks reduce toad hydration and breeding opportunities. Photo by Jonathan Webb. (C) A solar-powered cane toad trap, set up as part of a surveillance strategy to prevent toads from establishing on Groote Eylandt, Northern Territory. Photo by Deborah Bower. (D) Illustration of the effects of chemical suppression on tadpole growth. Pictured are two cane toad tadpoles from the same clutch: a control tadpole (left), and a tadpole that has been exposed to the suppressant chemical (right). Photo by Greg Clarke. See the online edition for a color version of this figure.

Fencing waterbodies is most useful in arid landscapes; in well-watered regions, fencing is unlikely to have measurable effects on toad numbers. Fencing is well suited to small-scale eradication attempts, but could also be used to exclude toads from large areas, provided that fences are maintained over multiple years (Letnic et al. 2015). Community groups comtemplating using fences for toad control might consider the use of more rigid fencing materials than the shade cloth fences used in previous research, although the impacts of such fences on native wildlife have not been studied. An alternative solution is to replace earthen dams with metal or plastic tanks (Figure 2B; Florance et al. 2011; Tingley et al. 2013). Providing that such infrastructure is well constructed and free of leaks, toads will not be able to access the water, and so will be unable to persist in the area. Replacing dams with tanks will be more expensive than fencing, and will require the participation and cooperation of landholders (Southwell et al. 2017). However, enclosed water storage tanks reduce evaporation, and thus present a potential benefit to pastoralists in arid regions.

TRAPS FOR ADULTS

For vertebrate pests, especially those with high reproductive rates such as cane toads, it is often strategic to remove reproductive females (Reidinger and Miller 2013), thereby reducing future population size. Male cane toads, like males of most anuran species,

make advertisement calls to attract females, and this aspect of their biology can be exploited to attract females to specific locations, including traps (Figure 2C). Recent research suggests that the call deployed on the trap can be engineered, using principles from studies of sexual selection in toads, to attract a higher proportion of females (calls attract both males and females), and to attract mostly gravid females (B. Muller et al., unpublished data). For example, in call choice experiments, female toads chose the largest male available that was smaller than themselves, and preferred calls with a low pulse rate and dominant frequency, whereas males did not show preferences for any particular call characteristics (Yasumiba et al. 2015).

Amphibian traps can also use lights to attract invertebrates as a food source (Schwarzkopf and Alford 2007). UV "black" lights work best to attract invertebrates to catch toads, as toads are repelled by white incandescent and fluorescent lights, but not by UV "black" lights (Davis et al. 2015). Traps with UV lights captured 10 times as many toads as those equipped with white fluorescent lights (Davis et al. 2015). Traps deploying both a toad call and a light were more effective than those using a call alone (Yeager et al. 2014).

As suggested for manual removal, trapping in the dry season can be an effective time to remove adults. Preliminary mark-recapture studies conducted with trapping at this time of year suggested that traps can remove up to 40% of the adult population (B. Muller, unpublished data). Future studies could determine whether traps can be used to eradicate extralimital incursions, or toad populations on very small islands.

Humane disposal of large numbers of trapped toads can pose barriers to the use of traps, as could inadvertent capture of nontarget (especially, native) species of fauna; however, thus far, adult toad traps have not captured any other species in Townsville, Queensland, or surrounds, after thousands of hours of trapping (L. Schwarzkopf, personal observation). In addition, any trapping program requires regular visits to traps to ensure that individuals are removed, and this can be challenging if traps are placed re-

motely. Methods to remove large numbers of toads humanely, and to monitor traps remotely, are in development. Traps should be available for commercial purchase in 2018.

TRAPS FOR TADPOLES

The high fecundity of cane toads (up to 30,000 eggs per clutch; Lever 2001), combined with their preference for anthropogenically disturbed habitats (González-Bernal et al. 2016), generates high levels of intraspecific competition. That competition may be especially intense during the larval phase, when large numbers of tadpoles compete for access to limited food resources in small waterbodies (Cabrera-Guzmán et al. 2013b). Recent research has revealed that cane toad larvae exhibit complex chemical communication systems, which appear to have evolved in the context of intraspecific competition. Toad tadpoles reduce the recruitment of subsequently laid clutches by actively searching out and consuming freshly laid toad eggs (but not the eggs of native frogs; Crossland et al. 2012). The tadpoles locate those eggs by following chemical trails, specifically toxins (bufagenins) that are released as eggs develop (Crossland et al. 2012). Funnel traps baited with toad toxins (obtained by squeezing toxins from the parotoid glands of adult toads) can attract and remove vast numbers of toad tadpoles from spawning waterbodies, with minimal collateral impact on native frog larvae (although potential impacts on other taxa, such as invertebrates and fish, warrants additional research; Crossland et al. 2012). To advance the development of a commercial tadpole trapping solution, the chemical attractant in cane toad eggs has been isolated and characterized, and a surrogate formation, prepared from adult toad parotoid secretion (BufoTab), has been evaluated and patented (R. J. Capon, A. Salim, R. Shine, and M. R. Crossland. "Chemical Attractant and Use Thereof, Patent." PAT-02059-AU-02. University of Queensland and University of Sydney, Australia, 2012).

Pilot studies, including those implemented by the public, contractors, and local government environment officers, have proved highly successful, with capture rates exceed-

ing 10,000 tadpoles per trap (R. Shine and R. J. Capon, unpublished data). The patent for tadpole trapping technology was recently licensed to a U.S.-based company, Springstar Inc., which will establish a Brisbane-based sister company to further develop, manufacture, and market a commercial product. In concert with this commercial approach, the University of Queensland has initiated a nationwide citizen science and community engagement program, the Cane Toad Challenge (http://canetoadchallenge.net.au), to build community awareness of cane toad control solutions, to marshal support, and to deliver tadpole trapping technology direct to the public.

Tadpole trapping is well suited to waterways abutting urban areas. The technology is cheap, semiautomated (set and leave), and with the provision of baits, easily and safely implemented by the public. Traps need to be checked daily, however, to avoid nontarget impacts on aquatic fauna, particularly native tadpoles. The long-term ecological benefit of tadpole traps has yet to be established, but remains a work in progress.

SUPPRESSION PHEROMONES

Intense competition between larval cane toads (discussed above) has resulted in the evolution of a species-specific chemical suppression system. As soon as they have transformed into free-swimming tadpoles, toad larvae begin to produce a substance that interferes with embryonic development of younger conspecifics (Crossland et al. 2012; Clarke et al. 2015, 2016). Eggs or hatchlings that encounter this substance exhibit severely reduced rates of growth and survival (Figure 2D). The substance has no detectable impact on the native frog species that have been tested to date, and thus may offer a species-specific suppressant of cane toad reproduction (Crossland et al. 2012; Clarke et al. 2015, 2016). Current studies are attempting to identify the nature of the suppressant substance, with a view to deploying it in natural waterbodies to prevent toads from breeding.

At present, evidence for the effectiveness of chemically mediated suppression is based primarily on laboratory work. The single field experiment to date reported that eggs were significantly suppressed after immersion in a pond containing toad tadpoles, but not after immersion in ponds without toad tadpoles (Clarke et al. 2016).

USING NATIVE SPECIES TO REDUCE TOAD NUMBERS

Laboratory studies have shown intense competition between cane toad tadpoles and the larvae of native frogs; in many cases the small, slow-swimming toad tadpoles are outcompeted by larger and more active tadpoles of native species (Alford et al. 1995; Alford 1999; Cabrera-Guzmán et al. 2011). Thus, we can suppress toad larval survival by encouraging native frogs to breed in the ponds used by toads, or by introducing eggs or tadpoles of native frogs directly (Cabrera-Guzmán et al. 2011, 2013b,c; Shine 2011). Similarly, many native invertebrates are voracious predators of toad tadpoles (e.g., water beetles and dragonfly larvae) or metamorph toads (ants), and we might be able to increase densities of such predators by manipulating habitat attributes (Ward-Fear et al. 2009, 2010a,b; Cabrera-Guzmán et al. 2012, 2013a, 2015b). Additionally, dense growth of vegetation around waterbody edges discourages toad breeding, such that replanting these areas might curtail toad recruitment (Hagman and Shine 2006; Semeniuk et al. 2007). Local government authorities in some parts of Australia insist on revegetation around potential toad-spawning sites following major building works, but there are no field studies of the effectiveness of this method for toad control. Many Australian predators also consume adult toads or parts thereof (typically leaving the parotoid glands uneaten), suggesting that high densities of native rodents (Cabrera-Guzmán et al. 2015a) and raptorial birds (Beckmann and Shine 2011) might also contribute to controlling toad numbers.

USING PATHOGENS FOR TOAD CONTROL

There are few documented cases of pathogens successfully controlling vertebrate pest populations; in all instances, they are introduced viruses controlling introduced

mammals (Shanmuganathan et al. 2010; Di Giallonardo and Holmes 2015). Important characteristics of a potential biocontrol agent include host specificity to the target organism and negligible impacts on native species (Simberloff 2012). In the case of cane toads, additional aspects of an effective control agent include self-dissemination and targeting of adults (Shanmuganathan et al. 2010).

Introduced species often lose many of the pathogens they carry in their native range (Blossey and Notzold 1995; Torchin et al. 2003) and this appears to be the case in cane toads (Barton 1997). Systematic surveys of cane toad pathogens have found that toads carry an array of potentially pathogenic organisms, but that few are associated with actual disease (Freeland et al. 1986; Delvinquier and Freeland 1988; Speare 1990; Freeland 1994; Speare et al. 1997; Hyatt et al. 1998; Linzey et al. 2003; Peacock 2006). Several known or potential pathogens (e.g., *Mucor, Ranavirus, Myxidium*) pose risks to native frog species. No promising candidate pathogens have yet been found that might widely suppress toad populations the way myxomatosis or rabbit calicivirus successfully suppressed feral rabbit populations (Shanmuganathan et al. 2010).

The finding that the lungworm infecting Australian cane toads was not Australian but rather a South American parasite (*Rhabdias pseudosphaerocephala*) brought with the founder toads, suggested a possible biocontrol mechanism (Dubey and Shine 2008). Initially, it seemed that strategically releasing the lungworm in advance of the toad invasion front might serve to infect and slow vanguard toads, which are typically parasite-free (Phillips et al. 2010b; Kelehear et al. 2011; Pizzatto and Shine 2012). Unfortunately, although the lungworm does not establish infections in most of the native frog species tested, it is deadly to one species, the magnificent tree frog *Litoria splendida* (Pizzatto et al. 2010; Pizzatto and Shine 2011). Thus, the use of *Rhabdias* as a biocontrol agent could have collateral negative impacts on native fauna. Evidence that toads infected with *Rhabdias* tend to disperse at greater, not lesser, rates than uninfected individuals also argues against its utility in slowing toad spread (Brown et al. 2016).

Toad populations often undergo dramatic declines that could plausibly be caused by an unidentified pathogen (Freeland 1986; Freeland et al. 1986; Speare 1990). Because toads congregate at high densities around shrinking waterbodies during the dry season, there is ample opportunity for pathogen transfer among toads (Freeland 1986). Thus, the search for new, toad-specific pathogens remains an important pursuit (Shannon and Bayliss 2008; Shanmuganathan et al. 2010).

Investigation into a recent (September 2014) mortality event in wild toads observed near Darwin in the Northern Territory has implicated an undescribed species of *Entamoeba* as the cause of death (C. Shilton, J. Slapeta, and G. Brown, unpublished data). Although this is the first pathogen identified to cause mortality in wild toads, further study is needed to ascertain where the toads acquired the organism, rates of infection and disease, the circumstances that make the organism pathogenic, and the risk it poses to native species.

Containment

Where eradication is not an option (as has so far proven to be the case for cane toads in Australia), the most powerful tool for minimizing the impact of an invasive species is to contain its spread (Epanchin-Niell and Hastings 2010). In most cases, this amounts to creating or maintaining barriers to natural spread. Such barriers may play out at several scales, from local asset protection (e.g., a wildlife park wanting to keep toads out of enclosures) to maintaining toad-free islands and keeping toads out of large areas of the Australian mainland.

The price of containment is eternal vigilance. A barrier that has been effective for years can be undone by a single colonization event comprising a male and female toad. It is currently unclear how commonly such colonization events occur, nor the primary modes by which they occur (although see Massam and Gray 2011). Toads sometimes colonize offshore islands if such islands are close to shore, if they are in the plume of a major river, and particularly in the monsoon tropics where large volumes of rain can leave freshwater lenses on seawater

that persist for weeks. Establishment events driven by accidental human introduction seem substantially rarer. Successful establishment in extralimital areas on the mainland, for example, is surprisingly rare (Lever 2001). Indeed, there are possibly billions of toads on the continent, and huge flows of traffic along the eastern seaboard where toads have been present for more than 85 years, yet there have been only three nonintentional successful colonization events recorded ahead of the main invasion front. All of these have been at the southern invasion front in New South Wales (one to Port Macquarie; one to Yamba; and one to Sydney; although smaller-scale introductions have occurred near Yamba; M. J. Greenlees, unpublished data). This low frequency of successful colonization events occurs despite a large number of individual toads being accidentally transported (White and Shine 2009). Such transportation events typically involve only a single animal (and so cannot establish a population); where they do involve multiple animals, the natural tendency of toads to segregate by sex (Zug and Zug 1979; González-Bernal et al. 2015) means that these multiple animals are quite likely to be all the same sex. The practice of conducting "toad races" as a tourist pastime in Queensland has, however, introduced toads to offshore islands with resorts (L. Schwarzkopf, personal observation).

Any containment strategy requires vigilant monitoring and a plan for rapid eradication if a small population is detected. Upon detection, the tools for local population reduction and eradication (above) can be brought into play, but successful eradication is more likely if an incursion is detected early. Below we review the new tools available for early detection of incursions, and sketch a promising containment strategy for mainland Australia.

ENVIRONMENTAL DNA SAMPLING

Environmental DNA (eDNA) sampling—the detection of genetic material released by organisms into the environment—can be a remarkably sensitive method for detecting freshwater species at low population densities (Thomsen et al. 2012; Smart et al. 2015). This sampling technique could, therefore, provide an early warning signal for new toad incursions on offshore islands or help identify extralimital populations on the mainland. Environmental DNA sampling may prove particularly effective when toads are congregated around waterbodies (e.g., in arid landscapes or during the dry season in more mesic environments).

To the best of our knowledge, no previous study has attempted to detect cane toad eDNA, but numerous studies have applied this approach to detect amphibians in wetlands and streams (Goldberg et al. 2011; Dejean et al. 2012; Thomsen et al. 2012; Pilliod et al. 2013; Rees et al. 2014). Furthermore, the wealth of genetic sequence data from across the cane toads' Australian range (Estoup et al. 2004; Rollins et al. 2015; Trumbo et al. 2016) coupled with the fact that there are no native toads in Australia, means that the development of suitably specific genetic primers should be straightforward. The key to implementing this sampling technique would be to identify likely cane toad breeding sites near locations with a high likelihood of human-assisted colonization (e.g., maritime shipping ports and airports on offshore islands; caravan parks, camping sites, and plant nurseries ahead of the mainland invasion front).

AUTOMATED ADVERTISEMENT CALL DETECTION

Male cane toad advertisement calls could be used to detect toad presence ahead of the mainland invasion front, or on offshore islands. Automated detectors that use computer algorithms to scan acoustic data for cane toad calls were designed and established prior to 1996 (Taylor et al. 1996), but both hardware and software for such tasks are improving rapidly, and new methods are constantly under development (Hu et al. 2009). The most useful versions of these methods automatically detect calls in the field, either in real time or with a short delay (less than one minute), and then upload that information to a server or telephone to allow an appropriate response.
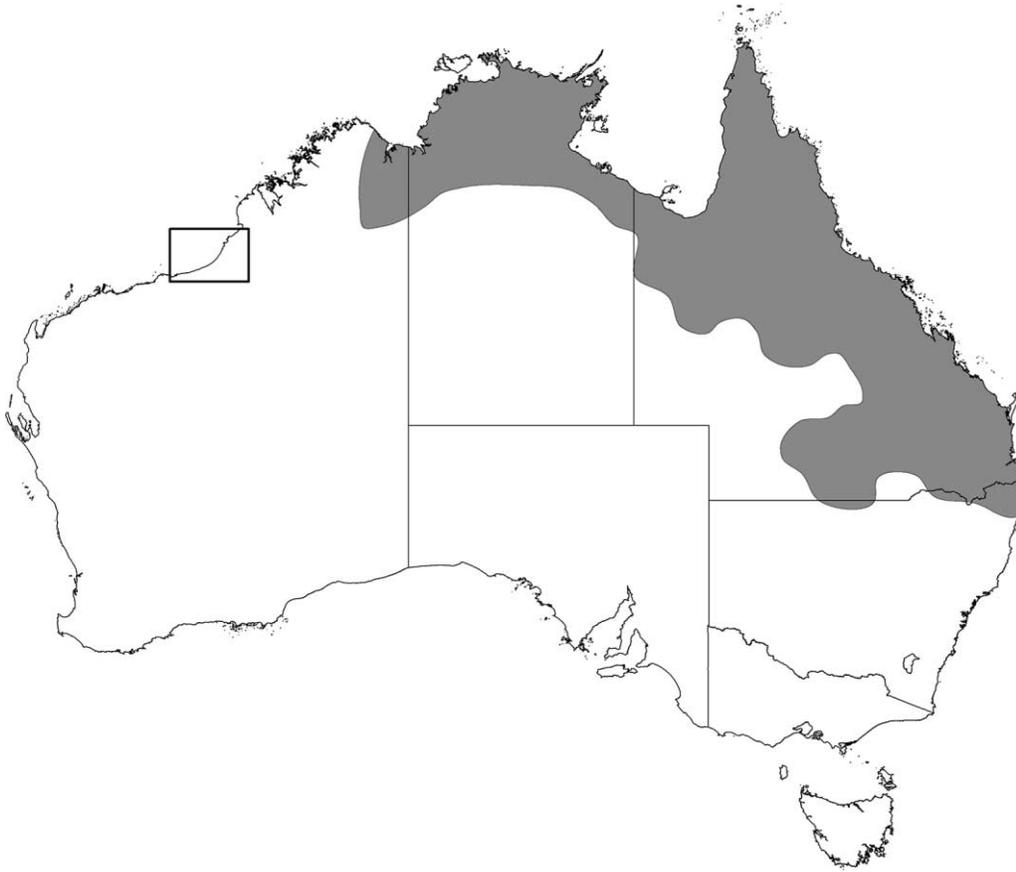
FIGURE 3. APPROXIMATE MAINLAND DISTRIBUTION OF CANE TOADS IN AUSTRALIA AS OF 2016
The box in Western Australia represents the approximate extent of the Kimberley-Pilbara corridor.

This approach successfully detected the cane toad front as it advanced across the Northern Territory (Grigg et al. 2006), but was plagued by equipment failure. More generally, detecting specific sounds in a natural setting using algorithms is a difficult computational problem because there are other sounds, such as wind, rain, and leaves fluttering, that can interfere with or mask animal calls, reducing the probability of detection. Detection algorithms must be tuned for their location of application and ground truthed to ensure effectiveness (Towsey et al. 2012). In addition, application of such systems in remote locations is hampered by high cost and inconsistent network coverage, which interferes with data upload. Acoustic detection would be best applied in locations at high risk of cane toad arrival, and in conjunction with other detection methods, including eDNA detection and, possibly, adult traps.

THE PILBARA LINE

In arid landscapes, cane toads rely critically on artificial watering points (AWPs), such as farm dams, for hydration and breeding (see the section Fencing Waterbodies; Florance et al. 2011; Tingley and Shine 2011). Thus, excluding toads from a large number of AWPs in an area ahead of the invasion front could potentially contain their spread. Florance et al. (2011) highlighted a promising area for such a strategy, between the Kimberley and the Pilbara regions in Western Australia (see inset of Figure 3). Here the Great Sandy Desert meets the coast, cre-

ating a chokepoint with very few natural perennial waterbodies. Simulation modeling of cane toad spread along this arid corridor suggests that the invasion front could be halted there by a waterless barrier approximately 80 km wide, for a total cost of about $4.5 million (Southwell et al. 2017). The benefit of such an action would be that we keep cane toads out of the Pilbara—an area with extremely high endemism—and 268,000 km^2 of the toads' potential range in Western Australia (Tingley et al. 2013).

The current proposal is to fit all AWPs with leak-free tank and trough systems (Figure 2B), with troughs that are inaccessible to toads (Southwell et al. 2017). This infrastructure could then be monitored by pastoralists (as part of their normal farm activities), and any leaks repaired as a conservation cost. Thus, we can engineer mutual benefit for both pastoralists and conservationists. This "Pilbara Line" represents an astonishingly cost-effective strategy to reduce the impact of toads and secure many of the last populations of toad-impacted predators, such as yellow-spotted monitors *Varanus panoptes* and northern quolls *Dasyurus hallucatus*. There is some uncertainty around the future of water use in the area of the Pilbara Line, with a government-subsidized push to increase the use of groundwater in the area for cropping; a move that would make the Pilbara Line substantially more expensive to implement. Other than this ongoing uncertainty around land use, the idea is very well developed: it needs an endowed trust fund to finance it and a small amount of research to design an effective tank and trough system.

genetic backburning

The waterless barrier required to stop toads at the Pilbara Line has to be approximately 80 km wide because invasion-front toads move astonishing distances: up to 60 km in a wet season. The reason they move such huge distances (an order of magnitude further than most other anurans move in their lifetime; Smith and Green 2005) is because toads on the invasion front have evolved to become hyperdispersive (Phillips et al. 2006, 2008, 2010a; Alford et al. 2009). When toads

were first introduced to Australia, they spread around 10 km per year; on the invasion front, they now spread closer to 50 km per year (Urban et al. 2008).

The vast difference in dispersal ability between invasion front and long-established toad populations suggests a radical idea for improving the effectiveness of the waterless barrier. What if, as the invasion front approached the Pilbara Line, we introduced toads from long-established populations on the nearside of the waterless barrier? This action would mean that we would be asking the barrier to stop the much less dispersive toads of the long-established populations (Alford et al. 2009) and, if the introduction was done correctly, the highly dispersive toads of the invasion front would never make it to the barrier. That is, for the very modest cost of introducing toads from Queensland ahead of the invasion front, we could make the waterless barrier substantially more effective.

This is a radical idea, and would likely face considerable community and political opposition. Nonetheless, early modeling work suggests that genetic backburning can substantially increase the effectiveness of landscape barriers (Phillips et al. 2016). Although more detailed modeling work is warranted, all of the evidence at hand suggests that—in the absence of unexpected surprises such as strong assortative mating between invasion front and range core animals—the idea should work.

Impact Mitigation

Even if attempts to reduce toad abundances (above) are successful, we are unlikely to eradicate toads completely over a large part of their range. Even low densities of toads may be ecologically catastrophic for native predator populations: a predator (such as a quoll or varanid) may be killed if it encounters a single adult toad (O'Donnell et al. 2010). As a result, substantial research has been devoted to an alternative tactic: to buffer the impact of toad invasion by changing the outcomes of encounters between toads and anuran-eating predators (O'Donnell et al. 2010; Ward-Fear et al. 2016). Other

researchers have focused on retaining the genetic diversity likely to be lost when predator populations collapse after toad invasion, and developing ways to restore that lost genetic diversity to wild populations postdecline.

conditioned taste aversion training

Smaller predators generally are unaffected by toad invasion at the population level, despite occasional mortality when toads are ingested (Shine 2010). Toxin content increases rapidly with toad body size, such that a small toad may not contain enough toxin to kill a predator that ingests it (Phillips et al. 2003). Instead, some predators that ingest small toads become ill, and rapidly learn to avoid toads thereafter (Webb et al. 2008, 2011; Somaweera et al. 2011; Ward-Fear et al. 2016)—a phenomenon known as "conditioned taste aversion" or CTA (Garcia et al. 1974). The reason that large predators (e.g., quolls, varanids, and freshwater crocodiles) are at high risk is that they attack large toads (which dominate the invasion front; Phillips et al. 2006) and thus are killed by their first encounter with a toad. If we could expose toad-naïve predators to small toads first, they might learn to avoid the lethally large toads when they arrive (Ward-Fear et al. 2016). Additionally, we could deploy "toad aversion" baits—sausages of toad flesh infused with an odorless, nausea-inducing chemical—ahead of the toad invasion front (Webb et al. 2008; O'Donnell et al. 2010; Price-Rees et al. 2013).

Laboratory and field trials with small "teacher toads" or toad-aversion baits have produced encouraging results. In three of the predator species most imperiled by toad invasion, CTA training has significantly increased survival rates. Initial studies on quolls (*D. hallucatus*) used captive-raised individuals that were later released into the wild (O'Donnell et al. 2010); studies on blue-tongued skinks (*Tiliqua scincoides intermedia*) used wild-caught individuals briefly retained in captivity for CTA learning trials (Price-Rees et al. 2011, 2013); and trials on yellow-spotted monitors (*V. panoptes*) exposed free-ranging lizards to small live toads. The pathway

of CTA learning in wild predators will likely differ depending on their physiology and ecology. This is a necessary consideration in the design of management strategies. For goannas, live toads were significantly more effective in buffering toad impact than were nausea-inducing sausages of toad flesh (which conferred no survival benefit; Ward-Fear et al. 2016).

The coordinated deployment of toad-aversion baits and "teacher toads" ahead of the toad-invasion front could help prevent local extinctions of numerous vulnerable species, such as northern quolls and goannas. Toad aversion baits could be aerially deployed in remote rocky habitats favored by quolls (O'Donnell et al. 2010), whereas teacher toads could be deployed on floodplains or near waterbodies frequented by yellow-spotted monitors (Ward-Fear et al. 2016). Teacher toads presumably have the potential to train a much wider array of fauna; their perceived benefit is, therefore, likely currently underestimated. However, the window of time to execute this strategy in the Kimberley region of Western Australia is rapidly closing. Field trials to determine which nontarget species consume toad-aversion baits are currently underway in this region (D. Pearson, personal communication).

It is important to note, however, that the CTA approach depends upon predators generalizing from small toads or sausages to large toads; the degree to which that condition is satisfied will depend upon the cues used for prey recognition. A sausage-trained predator that cues in on the visual stimulus offered by a large moving object may still attack a large toad, whereas one that cues in on chemical substances may avoid a large toad when it encounters one (provided that large live toads smell similar to a small toad or toad sausage). Northern quolls that were trained with small dead toads infused with a nausea-inducing chemical subsequently investigated and sniffed large toads when released back into the wild, but did not attack them (J. Webb, personal observation). Varanid lizards also rely heavily on chemical cues prior to attacking prey, so provided that the lizards become ill after ingesting a small toad or sausage, they may be less likely to attack a

large toad compared to a toad-naïve animal. Future work could usefully explore the nature of cues for prey recognition by potentially vulnerable predators, and hence the feasibility of prey-stimulus-generalization in CTA trials.

The largest implementation barrier to the deployment of aversion baits is their viability in the field. Nontarget species, such as ants and some bird species, may reduce bait availability; whereas extreme temperatures in northern Australia may lead to rapid bait disintegration and loss of cues. The largest hurdle to the release of teacher toads is likely to be public opinion. It is important, however, to remember that any mortality caused by teacher toads would have been inevitable once the toad-invasion front arrived; the benefits of this strategy, therefore, substantially outweigh the risks.

TARGETED GENE FLOW

Although toads have had major impacts on many Australian predators, populations of these predators can still be found in areas where toads have been present for more than 50 years. The persistence of predator populations in these long-colonized areas is often due to rapid adaptation to the presence of toads. These adaptations can be seen in predator morphology, but most powerfully take the form of behavioral avoidance of toads as prey (Phillips and Shine 2004, 2006; Llewelyn et al. 2014). If there is a genetic basis to toad-smart behavior, there is no reason why we should not be able to use targeted gene flow (Kelly and Phillips 2016) to introduce those genes into toad naïve populations. This can simply be achieved by breeding predators from long-colonized areas with populations soon to be impacted, or by using assisted reproductive techniques to move genes into populations via gametes (see section below), eliminating the need to move live animals long distances or across borders, thereby minimizing the transmission of unwanted pathogens. Potential outbreeding depression in native predators could be mitigated by sourcing individuals from populations that are climatically and ecologically similar to the recipient location.

GENOME BANKING AND ASSISTED REPRODUCTIVE TECHNOLOGIES

Permanent loss of genetic diversity is one of the greatest conservation challenges associated with declining populations (Frankham 2010). Although the ideal solution for managing declining populations is to arrest the cause(s) of decline, this is not always possible in the time frame required to prevent the erosion of genetic diversity. In such cases, genome banking and assisted reproductive technologies could play a pivotal role in preventing genetic diversity loss, and in reversing the effects of inbreeding and genetic bottlenecks through the production of live animals from genetic material stored prior to decline (Clulow and Clulow 2016).

The genetic rescue of wild populations has been demonstrated (Madsen et al. 1999), and reintroducing lost genes to wild populations from cryopreserved spermatozoa has been achieved for an endangered species (Howard et al. 2016). Madsen and colleagues demonstrated genetic rescue in a small, declining snake population through translocation of males from a separate population, resulting in a dramatic reversal of population decline (Madsen et al. 1999). Further, Howard et al. demonstrated that restoring lost genes to a population of endangered black-footed ferrets could be achieved by artificial insemination (AI), using cryopreserved sperm that had been stored for decades from the founders of a captive breeding population (Howard et al. 2016).

Encouragingly, work has already begun to develop protocols for collection and cryopreservation of varanid spermatozoa (one of the taxa most heavily impacted by toads, and a logical starting point for reptile genome storage; see Clulow and Clulow 2016) and on the AI procedures required to use cryopreserved sperm to restore genes. Targeted collection and storage of spermatozoa is a realistic goal in the short to medium time frame available before toads cause further loss of genetic diversity in many wild populations of predatory reptiles and marsupials (Clulow and Clulow 2016). Preliminary studies have successfully collected spermatozoa from live animals via electroejaculation, and

from animals postmortem. Optimization of these techniques in the near future will enable collection and permanent storage of spermatozoa from wild populations in advance of the toad-invasion front, in facilities that are now available in Australia and elsewhere. This material will then be available to restore genetic diversity postdecline via AI into wild females, potentially providing genetic rescue (Clulow and Clulow 2016).

One potential barrier to implementation of this approach is public and scientific resistance to genome storage and assisted reproduction for wildlife conservation. However, this resistance is greatly at odds with the generally positive attitudes displayed toward the same approaches in plants (i.e., seed banks, both for conservation and agriculture), in industry (horse, cattle, and livestock breeding and movement), and in human reproduction (e.g., sperm storage and in vitro fertilization). It is also at odds with the similar, albeit more costly and limited approach, of captive assurance colonies and reintroductions of live animals, often championed by conservation scientists and the public. This is likely a perception issue around the use of more sophisticated technologies (e.g., using artificial insemination with animals versus planting a seed in the ground), and should ease as these technologies become more widely used.

GENE EDITING AND GENOME ENGINEERING

Advances in DNA sequencing have enabled the generation of full genome sequences in reasonable time frames and at reasonable cost (PacBio sequencing; Rhoads and Au 2015). There is a current concerted effort to determine a full genome sequence for the cane toad (P. White, L. Rollins, and E. Holmes, unpublished data), which will provide the information needed to probe critical aspects of cane toad biology with gene editing.

In recent years, exciting new molecular technologies have emerged that enable the specific and directed editing of the genome of an organism (Gaj et al. 2013). The most widely used technique involves the Clustered Regularly Interspaced Short Palindromic Re-

peat (CRISPR) and the associated nuclease, CRISPR-associated protein 9 (Cas9). The CRISPR/Cas9 system is derived from a form of "adaptive antiviral immune mechanism" found in bacteria. It has been recoded for use in animals, and uses the nucleotide sequence complementarity of a short guide RNA to enable the Cas9 endonuclease to find, match, and cut the DNA of a target gene in an animal's genome. Cellular repair mechanisms, including homology-directed repair and nonhomologous end joining, can then be utilized and manipulated to either disrupt, remove, edit, or insert genetic traits with exquisite precision (Doudna and Charpentier 2014).

The CRISPR/Cas9 system has already been successfully applied to the western clawed frog *Xenopus tropicalis* to generate gene deletions that have provided insights into pigment biology (Shigeta et al. 2016). When adapting these methods to the cane toad, an obvious first approach would be to target a significant pathway of impact: the toad's toxin. As the structure, composition, and gene expression pathways of the toxins are revealed, key enzymes in the pathway to their production may be identified in the genome sequence, and these could be targeted using CRISPR/Cas9 to reduce or eliminate their production. Recent research (R. J. Capon et al., unpublished data) has identified one key enzyme that, if removed using a CRISPR/Cas9-mediated knockout approach, holds great promise for generating a toad that produces a nonlethal product from the toxin pathway.

The CRISPR/Cas9 system can also be used to assemble a so-called "gene drive" (see Esvelt et al. 2014 for details). At every reproduction event, the gene drive duplicates itself, and all offspring of that reproductive event will carry the gene drive (Figure 4; Esvelt et al. 2014). To this simple gene drive cassette other genes can be added (often referred to as "payload"). These ancillary genes would lead to a changed biological trait, e.g., reduced fitness (Esvelt et al. 2014), as they are pushed into the gene pool alongside the CRISPR/Cas9 cassette. Over a number of generations, the theoretical expectation is that all members of the
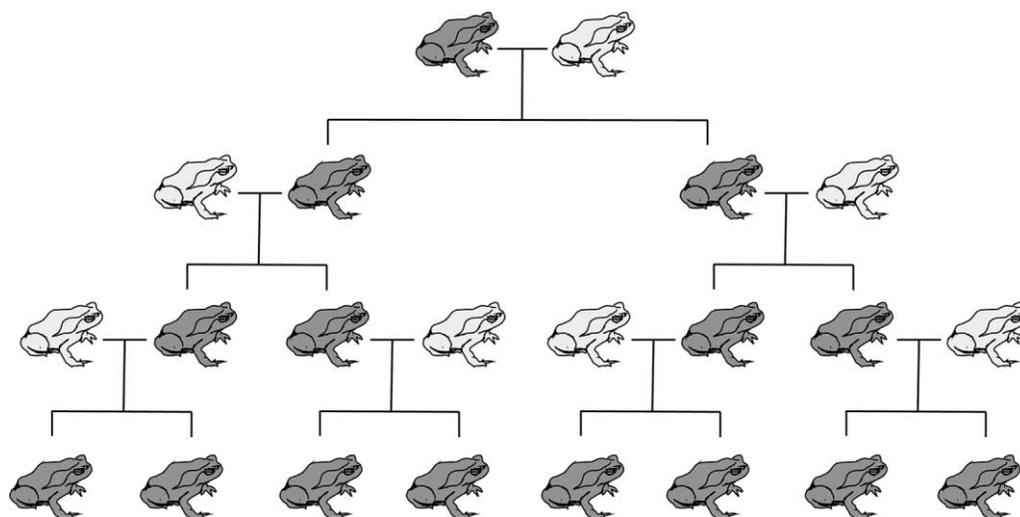
THE QUARTERLY REVIEW OF BIOLOGY

Figure 4. Spread of a Gene Drive in a Population by Sexual Reproduction
When a gene drive carrying animal (dark gray) mates with a wild type animal (light gray), all offspring will inherit the gene drive. This way the gene drive and its associated trait (e.g., reduced or no toxin production) can penetrate quickly into an entire population. Modified from Esvelt et al. (2014).

population will carry the modified trait. This outcome is expected even if that trait has a fitness penalty, providing it does not block sexual reproduction, on which it relies for propagation. The use of CRISPR/Cas9 gene drives is already well advanced in insects, where its scope is mainly in rendering disease vectors incapable of spreading human and veterinary pathogens (Gantz et al. 2015). More recently, gene drive approaches have been actively discussed for use in reducing pest animal populations and impacts (Esvelt et al. 2014).

The production and potential release of genetically modified (GM) animals would involve community engagement—to ensure that there is social licence to undertake deployment—and regulation and policy approvals from relevant state and federal governments. Engagement with indigenous communities on whose land the cane toad is impacting would be critical. All of this would need to be preceded by appropriate modeling of postrelease gene flow in target populations, and a thorough risk assessment. As transmission of gene drives is via sexual reproduction, there is no risk to nontarget species; however, the longer-term con-

sequences of releasing gene drives are yet to be understood. One risk is that an animal carrying a gene drive could accidentally or maliciously reach its native range (in this case South America). There is currently a very active international debate regarding how risks can be assessed and mitigated (Oye et al. 2014; Webber et al. 2015). However, while this debate continues, proof of concept work can be carried out using precision genome editing via CRISPR/Cas9, without the added gene drive function (e.g., to assess the viability of detoxified cane toads). With a few nucleotides removed and no added genetic material, such an organism might not be considered (or regulated) as a GM organism. If this is effective, a modification of this approach involving an integrated transgene (not a gene drive) to interfere with toxin production could be generated that would segregate and breed into the population as a dominant Mendelian genetic trait (such a toad would be regulated as GM). Each of these approaches could be steps to provide data for the evaluation and consideration of gene drive to push the "detoxified" trait into the wider toad population, such that the impact on key predators is all but removed.

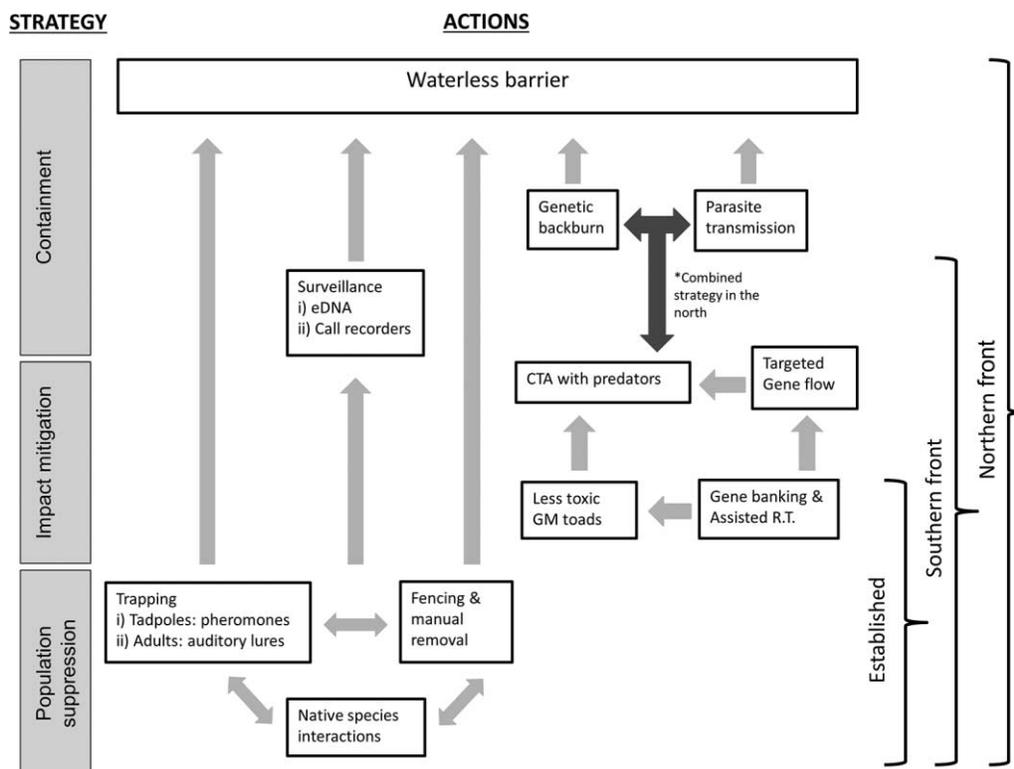**STRATEGY**   **ACTIONS**

Figure 5. Synergies Between Proposed Threat Abatement Strategies and Management Actions for Cane Toads in Australia

Each box represents an action that can be carried out independently under one of three broad strategies: population suppression, containment, and impact mitigation. Arrows depict directions of "synergies" (i.e., actions that can be achieved concurrently or an action that enhances the effectiveness of another). Brackets at the right group the management actions that can be applied synergistically within each of the three regions of cane toad occupation (nondispersive, established regions, and the dispersive invasion fronts of the North and South).

Genome engineering could be used not only to develop nontoxic toads, but could also be used to suppress cane toad populations. Genome engineering could, for example, enable the modification of genes controlling growth, physiology, life-stage transitions, or sexual development. This could empower functional studies of the biology of the cane toad in the search for its Achilles heel, and the pinch point through which a strong functional biological control might be exerted, some of which might not be genetic. Theoretically, there is a wide variety of approaches that can be used with a gene drive system, including biasing offspring sex ratios to eventually achieve reproductive population crashes or rendering a species susceptible to a lethal treatment (e.g., a prodrug) that is otherwise harmless to nonmodified animals or other species.

Synergies

Excitingly, many of the tools proposed in this paper create opportunities to design integrated management strategies that exploit synergies, thereby maximizing both effectiveness and resource efficiency (Figure 5). Below, we provide examples of integrated management strategies that capitalize on

some of these synergies, and discuss their applicability to invasion front versus long-established toad populations.

POPULATION SUPPRESSION

Although we currently lack the tools needed to eradicate toads from mainland Australia, we can substantially increase the effectiveness of local population control at waterbodies by implementing multiple tools concurrently. For example, fences could be erected around waterbodies to increase the effectiveness of manual removal of juveniles and adults. Survival and recruitment could simultaneously be reduced by trapping both adults and tadpoles, while applying suppressor pheromones and encouraging native predators of toads in and around waterbodies. This suite of tools could be applied across the toad's entire range, although fencing is more effective in arid landscapes than in landscapes with greater water availability.

CONTAINMENT

As outlined above, the effectiveness of the Pilbara Line at the northern invasion front could be enhanced substantially via genetic backburning. The effectiveness of this strategy also could be bolstered by reducing propagule pressure behind, or within, the waterless barrier, using population suppression tools. Similarly, population suppression tools could be applied to reduce propagule pressure near areas that are likely to serve as sources of island incursions (e.g., shipping ports). Some of these tools, such as adult traps and tadpole traps, could be coupled with containment tools, such as eDNA sampling and automated call detectors, to preemptively survey areas that are currently toad-free but at high risk of invasion.

Population reduction tools could also improve the likelihood of containment at the southern invasion front. In New South Wales, for example, satellite populations have been successfully eradicated using a strategy of sustained and systematic population reduction, i.e., a combination of adult and tadpole trapping, fencing waterbodies, and manual

removal (M. J. Greenlees and R. Shine, unpublished data).

Finally, the promise of new genetic technologies (CRISPR) is one of widespread control or mitigation. If such a promise is met, then it provides an endpoint to containment efforts. Containment need only work for as long as it takes us to develop powerful genetic techniques for toad control.

IMPACT MITIGATION

Conditioned taste aversion (CTA) with wild predators and targeted gene flow currently present the best options to mitigate toad impacts on native predators ahead of invasion fronts. These techniques could also be used to create "toad-smart" predators for reintroduction behind the invasion front, where predators were historically abundant. These predators could be produced from the same mainland populations using banked genetic stock and AI, enabling the restoration of predecline genetic diversity. Alternatively, predators for CTA training and reintroduction could be collected from offshore islands that are currently being kept toad-free via population suppression and/or containment methods.

Recent advances in genome engineering present additional synergies—if we can successfully reduce toad toxicity at all life stages, we could potentially engineer "teacher toads" with drastically reduced toxicity for release into the wild. Engineered toads would need to remain sufficiently unpalatable but nonlethal, such that toads of any size, including full-grown adults, could be used to "teach" predators ahead of the invasion front to avoid consuming wild-type (lethal) toads as they arrive in a new area. The benefits of this would be threefold: opportunities for learning in native predators would persist in the landscape for longer; risk of collateral damage to nontarget species would be diminished; and the "teacher toad" methodology would be more appealing to the public.

Gene banking and assisted reproductive technologies could further assist in the spread of engineered toad genotypes; sperm from less toxic toads could be banked to store and maintain lines for subsequent release into

wild populations. Samples of banked spermatozoa can be easily transported to any location and subsequently used to produce tens of thousands of offspring via IVF, a technology that exists for amphibians and has been applied globally (Clulow et al. 2012; Lawson et al. 2013; Clulow and Clulow 2016).

Conclusion

The cane toad's conquest of Australia is one of the best-documented examples of the wide-ranging ecological impacts of an invasive species. However, recent advances in our understanding of cane toad biology have led to the development and (in some cases) application of exciting new methodologies to suppress toad populations, contain their spread, and mitigate their ecological impacts. Although many of these methods are practical, some (such as CTA, the Pilbara Line, and genome engineering) may be controversial for certain groups among the general public. The obstacles to implementation thus involve the need for public engagement strategies to disseminate information to the public (in order to facilitate informed debate), as well as scientific issues. We need to clearly identify potential collateral risks of any control measures, and encourage public discussion of the way forward. Issues associated with genome engineering likely will require sustained discussion, whereas potential objections to the simpler methods (such

as release of "teacher toads") may well be amenable to rapid resolution.

When evaluating the strategies outlined here, the question should not only be, "what is the cost of trying?" but also, "what is the cost of doing nothing?" The cane toad system has attracted very detailed research, providing a diverse array of novel methods for invader control. For some of those methods (such as trapping and CTA induction), we have extensive field data to demonstrate effectiveness and lack of collateral impact. Now is the time for implementation: toads will have fully colonized the Kimberley within a decade.

More broadly, the Australian cane toad invasion illustrates the importance of understanding the fundamental biology of invaders, and how we can exploit that knowledge to develop effective new strategies for invader control and mitigation of invader impact. The cane toad example lends credence to the exhortation to know one's enemy; it is from such knowledge that creative strategies can spring. Investment in basic research on invader biology will, therefore, be a powerful strategy for success.

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