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Assessing the potential impact of invasive cane toads on a commercial freshwater fishery in tropical Australia

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Abstract

Context. The toxins produced by cane toads (*Rhinella marina*) are fatal to many Australian predators that ingest these invasive anurans. To date, the potential economic impact of the cane toad invasion has attracted little attention. Toads have recently arrived at a large impoundment (Lake Argyle) in north-eastern Western Australia, that supports a commercial fishery for silver cobbler (shovel-nosed catfish, *Arius midgleyi*), raising concern that the toads may inflict significant economic damage by killing fish.

Aims. Our research aimed to clarify the vulnerability of silver cobblers to the eggs and larvae of cane toads by determining (a) whether catfish are adversely affected if they prey on toad eggs or tadpoles, and (b) whether surviving catfish learn to avoid cane toad eggs and tadpoles in subsequent encounters.

Methods. We conducted laboratory feeding trials to examine feeding responses of catfish to cane toad eggs and tadpoles in early and late developmental stages. Fish that survived exposure to toad eggs and/or tadpoles were re-tested with potential prey of the same sizes and developmental stages four days later.

Key results. Our laboratory trials confirmed that some catfish eat toad eggs and die; but most catfish avoided the eggs. Catfish readily consumed toad tadpoles at both early and late developmental stages, but without experiencing mortality; and soon learned not to consume this toxic new prey type.

Conclusions and implications. Despite potential frequent episodes of mortality of small numbers of catfish during the wet season, the overall impacts of cane toads on the Lake Argyle fishery likely will be minimal.

Additional keywords: *Arius midgleyi*, Kimberley, Lake Argyle, *Rhinella marina*, silver cobbler.

Introduction

Invasive (alien) species that establish in natural or semi-natural ecosystems can threaten native biodiversity (IUCN-The World Conservation Union 2000; McNeely *et al*. 2001). In addition to posing a problem for species conservation, alien species can also inflict major economic damage on agriculture, forestry, stockbreeding, fisheries, road and water transportation, storage, water conservancy and human health (e.g. Xu and Ding 2003; Gutrich *et al*. 2007; Engeman *et al*. 2010). Invasive species can reduce the viability of native taxa via a variety of mechanisms, including competition, predation, habitat modification and pathogen transfer (Parker *et al*. 1999; Mack *et al*. 2000; Snyder and Evans 2006; White *et al*. 2006). One of the most direct and potentially damaging situations involves the arrival of an alien species that is highly toxic to native species, and is lethal if ingested. For example, some invasive plant species cause substantial mortality of livestock (e.g. Stiles 1942; DiTomaso 2000), and some invasive animals kill native predators that attempt to ingest them (e.g. Shine 2010).

The most intensively studied example of a toxic animal invader involves the spread of the cane toad (*Rhinella marina*) through tropical Australia (see review by Shine 2010). Native to South and Central America and Mexico, the cane toad has been introduced to more than 40 countries worldwide in misguided attempts at biological control (Lever 2001). The bufadienalide chemical defences of cane toads differ from the defensive chemicals produced by native Australian anurans, and many predators that attempt to consume cane toads die as a result (Lever 2001; Hayes *et al*. 2009). Cane toads have been implicated in rapid and sometimes massive population declines in a range of native predators, including snakes (*Acanthophis praelongus*; Phillips *et al*. 2010), lizards (*Tiliqua scincoides intermedia*; Price-Rees *et al*. 2010; *Varanus mertensi*, *V. mitchelli* and *V. panoptes*; Doody *et al*. 2009), crocodiles (*Crocodylus johnstoni*; Letnic *et al*. 2008) and mammals (*Dasyurus hallucatus*; O'Donnell *et al*. 2010). In other cases, even high rates of mortality of individual predators appear not to have translated into population-level impacts, especially in the case of predator species capable of learning aversion (Shine 2010; Brown *et al*. 2011).

Although most scientific attention has been paid to the impact of cane toads on terrestrial predators, the aquatic stages of the toad life-history (eggs and larvae) also possess potent bufadienalide toxins, and can cause substantial mortality of aquatic organisms (Crossland and Alford 1998; Crossland *et al*. 2008; Hayes *et al*. 2009; Greenlees and Shine 2011). The magnitude of impact from

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invasive cane toads will depend on a species' physiological resistance to bufadienalides, and whether or not the predators are able to detect the unpalatable nature of this novel prey type, and learn to avoid it in subsequent encounters (Crossland 2001; Nelson *et al*. 2011).

To date, the potential economic impact of the cane toad invasion has attracted little attention. However, in early 2009 the toads arrived on the eastern shores of Lake Argyle in the east Kimberley region of Western Australia, an area that is the site of Western Australia's only commercial freshwater fishery. This man-made lake was completed in 1972 by the damming of the Ord River. The fishery of Lake Argyle and the adjacent upper Ord River targets the shovel-nosed catfish or silver cobbler (*Arius midgleyi*, Ariidae) (Fletcher and Santoro 2008). Six commercial licence-holders take an average of 130 tons of this large (<1.4 m long, >10 kg) bottom-feeding fish species each year (Penn *et al*. 2005; Fletcher and Santoro 2008; Clark *et al*. 2009). However, catch rates are highly variable, and there are significant knowledge gaps relating to the target species' growth rates, longevity and mortality (Fletcher and Santoro 2008).

Since cane toads arrived at Lake Argyle in 2009, dead catfish (*A. midgleyi*, *A. graeffei*) have been found in the eastern part of the lake by us (R. Somaweera, pers. obs.) and by community groups (Kimberley Toad Busters, pers. comm.; Stop The Toad Foundation, pers. comm.). Plausibly, mortality may have been due to ingestion of toxic eggs or larvae of cane toads, because it has occurred at the sites where cane toads are known to have bred. The possibility of a major reduction in fish stocks due to toad poisoning has prompted concern from the local fishing community, and stimulated the present study. To clarify the vulnerability of silver cobblers to the eggs and larvae of cane toads, we conducted laboratory experiments to determine (a) whether catfish are adversely affected if they prey on toad eggs or tadpoles, and (b) whether surviving catfish learn to avoid cane toad eggs and tadpoles in subsequent encounters.

Materials and methods

Animal collection and husbandry

We collected 75 shovel-nosed catfish (mean total length ± s.e. = 156.4 ± 1.4 mm, range 127.0–211.7 mm; mean weight ± s.e. = 39.23 ± 0.66 g, range = 26–72 g) from the north-western part of Lake Argyle. This area has not yet been invaded by cane toads (based on our surveys), so we can establish responses to cane toad eggs and tadpoles before any opportunity for adaptive shifts (e.g. Phillips and Shine 2004, 2006) or aversion learning (e.g. Webb *et al*. 2008; Nelson *et al*. 2011). The fish were individually housed in aerated 70 L plastic bins (65 × 42 × 39 cm) filled with 50 L of untreated water from the lake, and acclimatised in captivity for five days, during which time they were fed daily with commercial fish food (catfish pellets, JMC, Derbyshire, UK).

We obtained cane toad eggs and tadpoles by inducing adult toads (collected from the Lake Argyle shores) to breed via subcutaneous injection of 0.25 mg mL^{-1} of leuprorelin acetate (Abbot Australia, Botany, NSW). Tadpoles were maintained in 70 L plastic bins filled with 50 L of lake water, and were fed on frozen lettuce *ad libitum*. All acclimation and experimental procedures were conducted in an outdoor shaded laboratory area (24−26°C water temperature).

Feeding experiments

After the catfish had spent five days in captivity, we exposed them to either 20 cane toad eggs, 2 early–mid stage cane toad tadpoles (Gosner 1960; developmental stages 25–36) or 2 late stage cane toad tadpoles (Gosner 1960; developmental stages 40–41). Toxin content is highest in the eggs, and lowest in the late stage tadpoles (Hayes *et al*. 2009) and these three aquatic stages thus represent a gradient of risk to aquatic predators. We used different fish to test responses to each developmental stage.

We conducted the experiments in the same 70 L plastic bins in which the fish were held. The bins were covered with mesh lids to exclude other predators (e.g. aquatic insects). Food was withheld from the fish for 24 h before the experiments began. Each fish was measured for total length, gape width and weight before the trials. After measuring the snout–vent length (for tadpoles) and determining stage of development (Gosner 1960), we introduced eggs or tadpoles to the bins at 1800 hours. For each experiment, 20 fish were exposed to toad eggs or tadpoles; 15 fish were used as controls. All controls received two pellets each of the usual commercial fish pellets they were fed before the trials.

After 14 h, we scored the eggs and tadpoles as untouched (eggs still attached as a string; tadpoles alive and unharmed), attacked (eggs separated from the gelatinous strand; tadpoles dead or damaged) or consumed (eggs or tadpoles missing); and we also scored the condition of the fish (dead or alive). At the completion of each experiment the uneaten eggs and tadpoles were removed and the fish was offered 10 food pellets to determine whether it was still prepared to feed. Fish that survived exposure to toad eggs and/or tadpoles were re-tested with potential prey of the same sizes and developmental stages four days later (i.e. 20 eggs or 2 tadpoles), to clarify whether or not individuals that attacked or consumed toads in the first trial avoided eggs and/or tadpoles on subsequent encounters.

The experiments described above involve potentially lethal effects, and for ethical reasons many authors have criticised this approach, advocating instead an assessment of sublethal impact. In cases where we need to assess the impacts of specific toxin levels for purposes of regulation and environmental management, it would indeed be preferable to use non-lethal assays. However, in the present case, the central question involves the magnitude of threat posed by an invasive organism, whose toxicity we cannot regulate. Thus, the issue is whether or not the invasion of toads is likely to have an ecological impact on the fish, not the exact level of toxin required for specific degrees of functional impairment.

This work was conducted under the approval of the Animal Ethics Research Committee of the University of Sydney (Approval No. L04/11–2010/3/5448) and permits from Department of Environment and Conservation of WA (Permit No. SF007728), Department of Fisheries of WA (Exception No. 1823) and Department of Agriculture and Food of WA (Permit No. 505).

Statistical analyses

We compared mortality rates of fish between control and toad-exposure treatments using Fisher's exact tests; when mortality occurred, we used logistic regression (Pearson χ^2) to examine the relationship between ingestion of toad stages and predator mortality. When fish consumed eggs or tadpoles during the first exposure and survived, we compared feeding responses between the first and second exposures using McNemar's tests; this analysis allows for the same fish being used in the initial and subsequent exposure to toads.

Results

Predation levels and toxicity

No fish in the control groups died during the trial period. Similarly, no fish died after consuming toad tadpoles: 17 fish ate 1–3 early–mid stage tadpoles (stages 25–36), and all 20 fish ate 1–3 late stage tadpoles (stages 40 and 41) without mortality or any overt effects on behaviour after 14 h. In contrast, ingestion of toad eggs proved fatal: the only two (out of 20) fish that ate toad eggs, both died in <14 h (Fig. 1). The low overall predation rate on eggs meant that survival rates did not differ significantly between the experimental and control fish (Fisher's exact test, $P = 0.50$), but ingestion of toad eggs was clearly linked to mortality of the predator (Pearson $\chi^2 = 20.0$, $P < 0.001$). All fish ate food pellets at the completion of the experiments, showing that any reluctance to consume toads was a specific response rather than a general reluctance to feed.

Learned avoidance

Because the only two fish that ate toad eggs died soon afterwards, we could not examine learned avoidance of toad eggs.

Catfish readily attacked toad tadpoles during the first trial, but largely avoided them during the second trial. The propensity to attack tadpoles declined between trial one and trial two for catfish tested with both early–mid stage tadpoles (McNemar's test, $P < 0.0001$) and late stage tadpoles (McNemar's test, $P = 0.0002$). The fish were also less likely to consume tadpoles in the second trial than the first (McNemar's test, $P < 0.0001$ for both early–mid stage and late stage tadpoles; see Fig. 2).

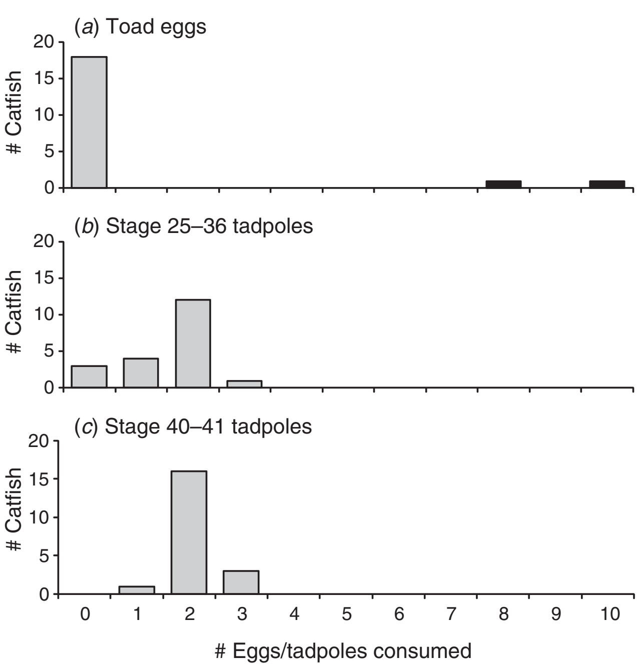

Fig. 1. Number of catfish that ate eggs or tadpoles of invasive cane toads in laboratory trials. Animals shown in black bars died as a result of consuming toad eggs.

Discussion

Fork-tailed catfishes of the family Ariidae occur throughout the shallow waters of the tropics (Nelson 1978; Kailola 1990) and half of the currently recognised 80+ species are restricted to the Australia–New Guinea region (Allen *et al.* 2003). Although several ariid species are economically important (Kossowski 1996; Teugels and Gourene 1998; Vidthayanon and Premcharoen 2002), previous studies of cane toad impact on Australian fish (e.g. Pearse 1980; Hearnden 1991; Crossland and Alford 1998; Wilson 2005; Greenlees and Shine 2011) have not included members of this widespread family. Broadly, however, the responses and vulnerabilities of silver cobbler to cane toads appear to be similar to those of previously studied fish species of other families.

Our laboratory trials with toad-naïve catfish showed that the risk of poisoning depends on which life-history stage of the toad is encountered by the predator; eggs pose a greater risk than tadpoles. This result mirrors the conclusions of Greenlees and Shine (2011), who suggest that the non-toxic jelly coat of eggs

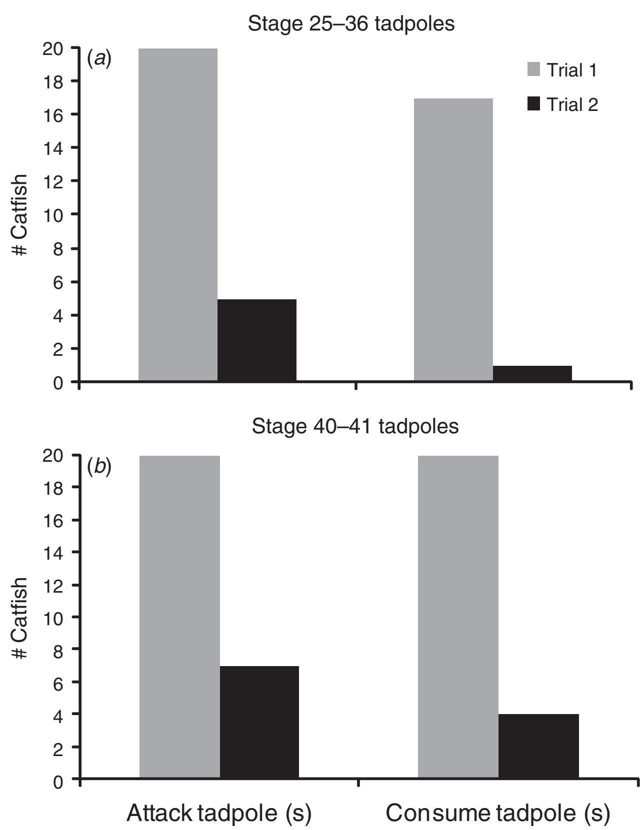

Fig. 2. Responses of shovel-nosed catfish to cane toad tadpoles as a function of previous exposure to these toxic anurans; the fish readily attacked and consumed tadpoles during the first trial but not during the second trial.

masks the toxins beneath, whereas the toxins of tadpoles are immediately detectable. Thus, fish can taste and reject tadpoles, and learn to avoid them (Crossland 2001; Nelson *et al*. 2011); whereas ingestion of eggs may prove fatal. In contrast, the larvae of native frog species do not appear to detect the toxicity of either eggs or tadpoles of the invasive toad, and thus do not learn to avoid them (Crossland *et al*. 2008; Hayes *et al*. 2009; Crossland and Shine 2010).

Ariids breed at the onset of the tropical wet season (Allen *et al*. 2003), which coincides with the breeding season of cane toads in the Lake Argyle area. Fork-tail catfish display intensive parental care involving oral incubation and mouth brooding (Kailola 1989). Brooding males of *A. midgleyi* form groups in deeper water, where the adults also feed (Allen *et al*. 2003). Hence, the life stages of catfish that are most likely to forage in shallow water, and thereby encounter toad tadpoles and eggs, are juvenile fish similar in size to those we used for the current study. Even for this size class, the frequency of encounters between catfish and toads is reduced by:

(1) selectivity in spawning-site selection by cane toads (e.g. based on pond side vegetation density: Hagman and Shine 2006; Semeniuk *et al*. 2007) resulting in strong spatial concentration of toad breeding;
(2) the toads' preference for shallow water for spawning, apparently reflecting dangers to female toads that venture into water deep enough that they can be drowned by the weight of amplexing males (Bowcock *et al*. 2009); and
(3) the brief duration of the egg stage in cane toads (<48 h: Crossland and Alford 1998), such that these potentially lethal food items are available only briefly.

The end result is that toad eggs are likely to be encountered by only a small proportion of catfish within the population. Encounter rates of fish with toad tadpoles will be much higher and more prolonged (because the tadpoles venture into deeper water, and the stage can last for >4 weeks: Lever 2001) but such encounters pose minimal risk to the fish.

Our results showed that shovel-nosed catfish can consume small numbers of toad tadpoles without ill effect, but then tend to avoid them during subsequent encounters. Presumably, this avoidance is a result of unpalatability. Because we did not continuously observe the fish during feeding trials, we do not know whether or not they showed any overt short-term signs of distress after ingesting tadpoles. In feeding trials, barramundi (*Lates calcifer*) and sooty grunters (*Haphaestus fuliginosus*) spat out cane toad tadpoles immediately after biting, and displayed signs of distress (vigorously opening and closing their mouths and shaking their heads from side to side for up to a minute: Crossland 2001). Like the catfish that we studied, barramundi (Crossland 2001) and the northern trout gudgeon (*Mogurnda mogurnda*) (Nelson *et al*. 2011) exhibit learned avoidance of toad tadpoles after an initial encounter.

The impact of cane toads on the catfish of Lake Argyle may also involve predation on adult toads. *Arius midgleyi* is omnivorous (Allen *et al*. 2003). In a study at the adjoining Lake Kununurra (Doupé *et al*. 2003), the diet of *A. midgleyi* (length range 273–900 mm TL) comprised fish (~42%), insects (~12%), prawns (cherabin, ~11%), bivalves (~10%), crayfish (redclaw, ~8%) and plant matter (~6.5%). Analysis of 39 stomach samples of *A. midgleyi* (length range 430–765 mm TL) from southern Lake Argyle (where toads were not present at the time of study) yielded bony bream (*Nematalosa erebi*), lesser salmon catfish (*A. graeffei*), belostomatid beetles and plant material (R. Somaweera, unpubl. data). However, a juvenile cormorant and water rats have been found in the stomachs of large (>1 m) silver cobblers caught in Lake Argyle (J. Williams, pers. comm.) and a hatchling freshwater crocodile was found in a stomach of *A. midgleyi* from Lake Kununurra (G. Allen, pers. comm.). Hence, adult catfish inhabiting deeper water may attack prey on the surface. Such prey potentially could include adult cane toads, which occasionally swim in open waters at Lake Argyle over 1 km from shore (Scott-Virtue 2010). However, we have not found cane toads in the stomachs of any of the six large catfish (mean total length = 972 mm) that we have so far found dead in Lake Argyle.

Although our data suggest that invasive cane toads will indeed cause mortality to the commercially important silver cobbler in Lake Argyle, the effects are likely to be minor. In keeping with this prediction, there are relatively few records of fish being killed by toad ingestion in tropical Australia – especially, relative to the very high toad-induced mortality rates reported for some other native taxa, including tadpoles of Australian native frogs (Crossland *et al*. 2008; Crossland and Shine 2010; Shine 2010). Grace and Sawyer (2008) reported ~70 spangled perch (*Leiopotherapon unicolor*) thought to have been killed by ingesting toad eggs or tadpoles, and a community group (The Kimberley Toad Busters) reported two large dead catfish at Coolibah Station (Scott-Virtue 2005*a*) and many dead 'baitfish' in creek systems containing cane toad eggs and tadpoles (Scott-Virtue 2005*b*). Mortality of salmon catfish (*Arius graeffei*) in and around Matilda Creek on the eastern side of Lake Argyle was also attributed to cane toads by this community group, but the possibility of by-catch from the fishing industry cannot be ruled out in this case. We expect that cane toad invasion of Lake Argyle will result in frequent episodes of mortality of small numbers of catfish during the wet season, mostly in shallow water, but that the toads will have far less impact on this commercial fishery than on the native terrestrial fauna living in the same area.

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