

Artificial water points facilitate the spread of an invasive vertebrate in arid Australia

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Summary

1. The spread of invasive species after their initial introduction is often facilitated by human actions. In some cases, invaders only become established in habitats where dominant native species have been displaced as a result of human actions or where humans inadvertently provide essential resources such as food, water or shelter.

2. We investigated if dams that provide water for livestock have facilitated the cane toad's (*Rhinella marina*) invasion of a hot semi-arid landscape by providing toads with a resource subsidy and hence refuge from extreme heat and aridity. To determine the relationship between the presence of surface water and habitat occupancy by toads, we surveyed natural and artificial water features for cane toads during the annual dry season. We used radiotracking and acoustic tags to determine whether movement patterns and shelter use of cane toads were focussed around dams. To determine whether dams provide toads with refuge from extreme heat and aridity, we deployed plaster models with internal thermometers to estimate ambient temperatures and toad desiccation rates in shelter sites. To determine whether dams alleviate the stress experienced by toads, we measured plasma corticosterone levels of toads that sheltered in and away from dams.

3. Toads were present in sites with standing water and absent from waterless sites. Most radiotracked toads sheltered within 1 m of water. Toad movements were focussed around water. Toads tracked with passive acoustic telemetry over a 6-month dry season were highly resident at dams.

4. Plaster models placed in toad shelter sites away from the water lost 27% more mass and experienced higher temperatures than models placed near the water's edge. Toads that sheltered in terrestrial shelters exhibited higher plasma corticosterone levels compared to toads that sheltered near dams.

5. Dams provide toads with refuge habitats where they are less at risk from overheating and dehydration.

6. *Synthesis and applications.* Artificial water points can facilitate biological invasions in arid regions by providing a resource subsidy for water-dependent invasive species. Our study suggests that there is scope to control populations of water-dependent invasive vertebrates in arid regions by restricting their access to artificial water points.

Key-words: artificial water, invasion hub, invasive species, physiological stress, resource subsidy, *Rhinella marina*

Introduction

The spread of invasive species after their initial introduction has often been facilitated by human actions, such as habitat modification or inadvertent dispersal by

humans (Didham *et al.* 2005; White & Shine 2009). In some cases, invaders only become established in habitats where dominant native species have been displaced as a result of human actions or in commensal situations where humans inadvertently provide essential resources such as food, water or shelter (Didham *et al.* 2007). The often tight linkage between human actions and the spread and impacts of invasive species has led some biologists to

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question whether invasive species are drivers or passengers of environmental change (MacDougall & Turkington 2005).

Scarcity of water and extreme heat constrain biological activity in arid environments and are crucial factors determining both the distributions of organisms and human economic activity (Whitford 2002; Mudd 2010; Smith 2013). Vertebrate species that live in deserts display a wide range of physiological and behavioural adaptations to maintain their water balance and body temperature within tolerable limits by regulating exchanges of heat and water (Whitford 2002). Some arid-dwelling vertebrates possess physiological mechanisms to reduce water loss and metabolic rate. Other species evade heat and minimize water loss by restricting periods of activity and seeking shelter in microhabitats that are cool and/or moist. Although many desert vertebrates obtain all or most of their water requirements through their food or store water during periodic rainfall events, other animals that live in arid environments must have regular access to water (Withers 1998; Whitford 2002; Dawson *et al.* 2006).

Humans' increased ability to capture and redistribute scarce water has been a key driver of economic growth in arid regions (James, Landsberg & Morton 1999; Kingsford 2000; Mudd 2010). Livestock grazing is an important land use in arid lands, but is frequently constrained by scarcity of surface water as animals such as horses, cattle, sheep and goats must drink regularly (Fensham & Fairfax 2008). To increase the livestock carrying capacity of arid rangelands, pastoralists often create artificial water points (AWP) where water is provided to animals via troughs or dams (Perkins & Thomas 1993; James, Landsberg & Morton 1999). The presence of AWP has substantially increased the availability of standing water and spatial distribution of water in rangelands (Fensham & Fairfax 2008). For example, in Australia, the creation of AWP

over the last 150 years means that there are now few places more than 10 km from water across the approximately 70% of the continent that is used for livestock grazing (James, Landsberg & Morton 1999). By way of contrast, prior to European settlement, surface water was rare in this low rainfall region and normally occurred only in the channels of major rivers and isolated springs and was only widespread for brief periods following rain.

Water is a limiting factor for many species; therefore, modification of hydrological regimes by humans can have significant impacts on the structure and function of arid ecosystems (Perkins & Thomas 1993; Stromberg, Tiller & Richter 1996; Kingsford 2000). By providing a resource subsidy for animals that must drink, AWP are thought to have facilitated range expansions and/or population increases in native and invasive animals in Australia's arid rangelands (James, Landsberg & Morton 1999; Dawson *et al.* 2006) and can provide previously unavailable habitat for aquatic organisms (James, Landsberg & Morton 1999).

In this study, we examine how the redistribution of water in a semi-arid landscape can provide a resource subsidy and subsequently influence the distribution and behaviour of an invasive species. Our study area was the Victoria River District of the Northern Territory. This semi-arid region has a vast network of bore holes which supply water to small dams which in turn supply cattle drinking troughs (Figs 1, 2). The study area is currently being invaded by the cane toad *Rhinella marina* L., a non-arid-adapted anuran from tropical America that is toxic to many native Australian predators (Shine 2010). Consequently, the invasion of cane toads has precipitated dramatic population declines in some predator species that frequently consume anurans (Letnic, Webb & Shine 2008; Doody *et al.* 2009). Our specific aims were to: (1) determine whether the activity of toads is concentrated around

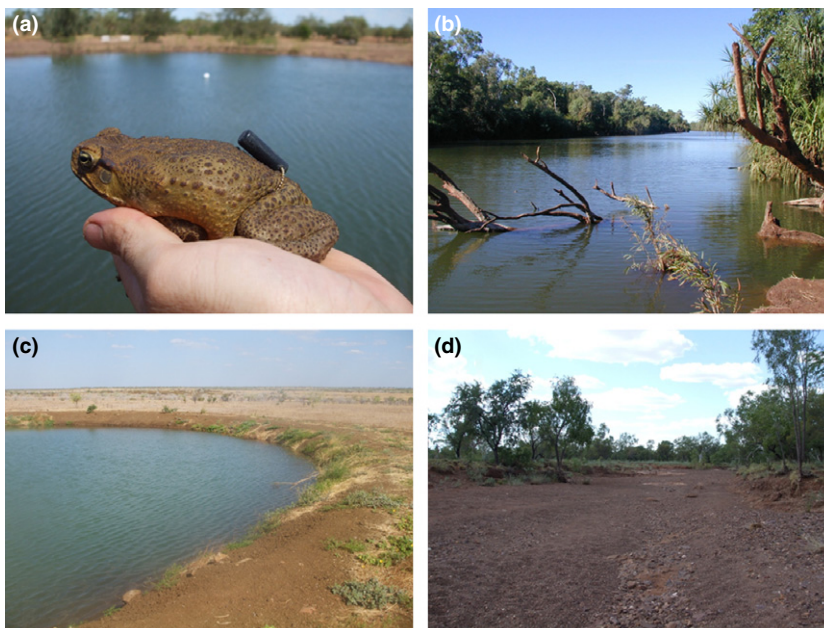


Fig. 1. (a) A cane toad fitted with chain-link waistband and acoustic transmitter. (b) A typical natural waterhole on the Victoria River during the dry season. (c) A typical dam to provide water for livestock. (d) A dry creek bed.

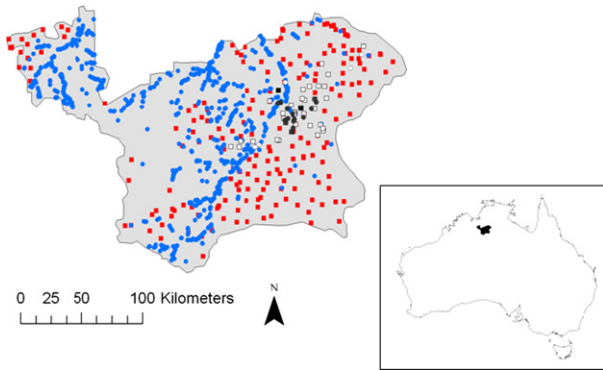


Fig. 2. The catchment of the Victoria River, Northern Territory, Australia, where average annual rainfall <700 mm. Black circles represent surveyed dry natural water holes, white circles represent surveyed wet natural waterholes, black squares indicate surveyed dry artificial water points, and white squares indicate surveyed wet artificial water points. Red squares represent other artificial water points located throughout the catchment, and blue circles represent other natural waterholes.

sources of permanent water and (2) examine how dams influence toads' movements, water balance and physiological stress.

Materials and methods

STUDY SPECIES

The cane toad has spread through more than 1 million km² of Australia since its introduction to Queensland in 1935 (Shine 2010). This spread has resulted from both range expansion overland and along watercourses (Phillips *et al.* 2007) and from inadvertent human-assisted dispersal (White & Shine 2009). Unlike most native Australian desert-dwelling frogs, which possess physiological adaptations (cocoon formation, aestivation, reduced metabolic rate) to survive long dry periods (Withers 1993, 1998), cane toads cannot physiologically control evaporative water loss through their skin (Tracy *et al.* 2007). Thus, toads are susceptible to dehydration throughout their life cycle, but become increasingly resistant to dehydration as their body size increases due to a decrease in their surface-area-to-volume ratio (Seebacher & Alford 2002; Child *et al.* 2008). To combat dehydration, adult cane toads are active nocturnally and select moist, cool microhabitats as shelter sites during the daytime (Schwarzkopf & Alford 1996; Seebacher & Alford 2002). The arid regions of northern Australia are characterized by distinct wet and dry seasons, with almost all annual rainfall occurring within a brief wet season (December–March). During the dry season, high desiccation rates and limited moisture availability restrict cane toads to microhabitats near permanent water (Brown, Kelehear & Shine 2011).

STUDY AREA

Our study area lies in the Victoria River catchment (34 462 km²), Northern Territory, Australia, and receives <700 mm of annual rainfall. Mean annual rainfall at the southern margin of the catchment is approximately 500 mm per annum (Australian Bureau of Meteorology). Cane toads first invaded the northern

part of the study area in the wet season of 2006–2007 (Letnic, Webb & Shine 2008) and are expanding their range westwards and southwards into increasingly arid regions. The Victoria River catchment experiences a semi-arid, monsoonal climate characterized by a hot humid wet season (December–March) and a hot dry season (April–November). Temperatures are high year-round and, on average, exceed 30 °C on 286.4 days each year at Wave Hill (17.45°S, 130.84°E).

In most years, no rain falls between April and September, and most stream flow occurs during the wet season (December–March) when monsoonal rains bring > 80% of the annual precipitation. During the late dry season (August–November), natural sources of water are limited to a small number of permanent natural springs and disconnected pools in the Victoria River and its major tributaries and are particularly scarce in the eastern and south-western parts of our study region (Figs 1b, 2). During the study, no rain fell from March to November in 2009, while the 2010 dry season experienced two rainfall events in early May (44 mm) and July (5 mm; Australian Bureau of Meteorology).

The major landforms in the catchment are undulating plains dominated by Mitchell grasses *Astrelba* spp. During the dry season, deep cracks form in the soils due to shrinkage owing to water loss. Permanent waterholes on river channels are lined with tall trees (10–20 m height) such as *Melaleuca viridiflora*, *Eucalyptus camaldulensis*, *Nauclea orientalis* and a dense understorey of *Pandanus aquaticus* along the margins of the water (Fig. 1b). The vegetation of ephemeral creek lines (Fig. 1d) is dominated by *Eucalyptus* spp. and *Acacia* spp. up to 5 m tall with an understorey of grasses. Other vegetation types interspersed through the landscape include *Triodia* grasslands on lateritic soils and limestone hills.

Commercial cattle grazing has been conducted throughout the study area since the late 19th century. Water is a limiting resource for grazing livestock in this hot semi-arid region. To increase the amount of grazing land available for cattle, pastoralists have established AWP at intervals of 5–10 km throughout the landscape (Figs 1c, 2). There are two types of AWP within the study area. Most are circular shaped above-ground dams, approximately 30 m in diameter with walls constructed of earth into which water is pumped by diesel-powered bores or windmills (Fig. 1c). The earthen dams serve as reservoirs which provide water to livestock via gravity-fed troughs fitted with a float valve (see Appendix S1, Supporting Information). In some cases, the reservoirs are above-ground tanks constructed of plastic or steel (see Appendix S1, Supporting Information) which provide water to livestock via gravity-fed troughs in the same way as dams. Cane toads cannot normally access the water held in troughs as they are too far above the ground (typically >50 cm) for them to jump into.

TOAD OCCUPANCY AT WATER FEATURES

We conducted timed active searches for cane toads at 27 natural and 33 artificial water features in September–October 2009 within the range of cane toads, following the protocol of Bradford *et al.* (2003). We classified water bodies as wet if standing water was present at ground level, and dry if no standing water was present at ground level. For example, sites with tanks that fed water into a trough were classified as dry, if there was no water on the ground and the trough was greater than 50 cm above the ground level. Survey sites were identified with 1:250 000-m scale maps and pastoral maps indicating artificial water points and natural

drainage features. The timed toad surveys consisted of a search for adults, juveniles and tadpoles along a 150 × 10 m transect along the margins of water features and in the water where present, by three individuals for a maximum of 15 min (45 person minutes). We searched all sites by day and again at night if no toads were detected during the day. Night surveys were conducted using 50-Watt hand-held spotlights. In all surveys, we searched for active toads on the ground or in the water (by snorkelling), and for inactive toads by inspecting under grass tussocks, using an inspection camera or torch to look into cracks and burrows and by turning cover items such as logs and large rocks. During daytime surveys, toads were observed at 60% (36/60) of sites. Night-time surveys were conducted at 24 sites where toads were not located during daytime surveys.

DIURNAL SHELTER SITES USED BY TOADS AROUND DAMS

We used radiotelemetry to identify the diurnal shelter sites of toads. We fitted radiotransmitters to 5–7 adult (range: 80–120 mm SUL) toads at each of six dams ($n = 39$ total) over four-day periods in September and October 2009. Toads were captured at night at the water's edge between 20:00 h and 22:00 h. On capture, the toads were fitted with a radiotag (Sirtrack, New Zealand; mass = 3 g, dimensions, 27 × 14 × 8 mm, <3% of toad mass) attached to a metal chain-link waistband (Brown, Kelehear & Shine 2011) and released at their capture site. Toads were tracked to their daytime shelter sites between 09:00 and 12:00 h each day for four consecutive days post-release. Toads were not disturbed during tracking. For each shelter site, we recorded the habitat type and distance to water (m).

WATER USE AND LONG-TERM RESIDENCY OF TOADS AT DAMS

We used acoustic telemetry to determine water-use patterns by toads at two dams over a 6-month period throughout the dry season from April 24 to 18 September 2010. Both dams were roughly circular (diameter = approx. 30 m; max. depth = 2.5 m) with little riparian or aquatic vegetation.

At each of the two dams, we captured 10 adult cane toads (five males and five females) for acoustic telemetry and recorded their mass and snout-urostyle length (SUL). Males ranged from 97–116 mm SUL (mean ± SE = 109 ± 3) and 165–230 g (195 ± 15), while females ranged from 86.5–132 (mean ± SE = 120 ± 4) and 160–340 g (276 ± 17). We fitted a coded acoustic transmitter (T-9, Thelma Biotel, Norway; length: 16 mm; weight in water: 1.8 g; frequency: 69 kHz; ratio of transmitter mass in water to toad mass = 0.5–1.0%) to each toad with a metal chain-link waistband (Fig. 1a).

The acoustic transmitters emitted unique coded signals so that each toad could be individually recognized. Signals from the transmitters were detected with passive listening stations (VR2Ws, VEMCO, Canada) suspended beneath a float at 1 m depth in the middle of the dam. Listening stations only detect signals from acoustic tags through water; thus, the toads had to be present in the water for a signal to be detected (e.g. Fig. 1b). Once a toad left the water, the signal was lost. Because acoustic signals transmit better in freshwater than in saltwater and the distance from the water's edge to the centre of the dam never exceeded 12 m, it is likely that signal detection probability was

high. We defined a visit as a period of detection that commenced when a toad was first detected in the water and finished if no signal had been detected thereafter for 30 min. In this way, we determined the number and duration of visits to the dam by toads.

MODEL DESICCATION RATES AND TEMPERATURES INSIDE TOAD SHELTER SITES AT DAMS

We estimated temperatures and desiccation rates in shelter habitats utilized by radiotracked toads using plaster blocks (80 × 58.6 × 25 mm) containing an internal i-button® (Maxim, San Jose, CA, USA), temperature logger. Saturated plaster blocks have similar dehydration rates to live toads (Tracy *et al.* 2007). Rates of water loss and temperatures of plaster blocks were determined by deploying blocks in three habitats: partially submerged at the water's edge, under grass and in soil cracks. Prior to deployment, models were submerged in water for 24 h and weighed. Plaster models were deployed at two dams (Dry bore and N48) from 13–15 October 2009. At each dam, three models were placed in each of the three habitats. Models were deployed for 48 h and then re-weighed.

For each model, we calculated mean and maximum temperatures recorded by the internal i-button over the 48-h period, and the number of hours during which temperatures exceeded 35 °C. We chose this temperature because previous studies have shown that cane toads begin experiencing spasms above 34.3 °C (Johnson 1972); hence, toads may have experienced some thermal stress at temperatures >35 °C.

PHYSIOLOGICAL STRESS RESPONSES IN SHELTER HABITATS

To compare the effects that sheltering in dams or terrestrial shelters had on the physiological stress experienced by toads, we measured plasma corticosterone level. Previous studies have linked elevated levels of corticosterone, in cane toads and other amphibians, to increased physiological stress and lower survival and have thus used plasma or urinary corticosterone levels to measure the sublethal effects of environmental stressors (Jessop *et al.* 2013; Narayan 2013). We collected plasma samples from 30 toads divided equally into individuals that resided in terrestrial shelters ($n = 15$) or at the water's edge in dams ($n = 15$). We collected blood samples at dusk, which coincided with the first arrivals of toads moving from their shelters to dams to rehydrate. To distinguish between toads that had sheltered in terrestrial shelters or dams, we simply intercepted 'terrestrial' toads on arrival at the dam. We then simultaneously collected blood samples from both habitat groups. Toads were euthanized on capture using rapid cervical dislocation. We collected 2 mL of blood within 30 s following decapitation. This brief sampling duration between capturing, handling and obtaining blood samples from toads is well below the 3- to 5-min range necessary to induce further stress-related increases in toad plasma corticosterone levels (Narayan 2013). Blood samples were placed into lithium heparin vials and stored on ice for <1 h before being centrifuged at 5037 g for 5 min and the plasma separated. Samples were stored at –20 °C until they were assayed. Body mass and body length were measured to estimate each individual's hydric state using residual scores derived from a log mass to log length linear regression model (Jessop *et al.* 2013).

Total plasma concentrations of corticosterone were measured using a commercially available ELISA kit (Cayman Chemical, Ann Arbor, MI, USA). Following the kit's methodological protocols and using 10 μ L of twice extracted plasma, final corticosterone concentrations were calculated from standard curves and then corrected for individual sample recovery and addition of 3H-corticosterone (Jessop *et al.* 2013). Average extraction efficiency for corticosterone was $86 \pm 1\%$. Intra- and interassay coefficients of variance were estimated at 5% and 11%, respectively.

STATISTICAL ANALYSES

We tested the hypothesis that radiotracked toad individuals were more likely to occupy diurnal shelter sites in the water than away from water using Cochran's Q test (Sokal & Rohlf 1981). We used two-way analysis of variance (ANOVA) to examine the effects of dam (N48, Dry Bore) and habitat (water, grass, crack) and their interaction on proportion of mass lost by plaster models after 48 h of deployment, the mean temperature and maximum temperature experienced by models over the 48-h deployment, and the number of hours during which temperatures of the models exceeded 35 °C over the 48-h deployment. Data on proportion of mass lost were arcsine-transformed prior to analysis.

To determine whether plasma corticosterone levels of toads varied between toads that sheltered in the water or terrestrial habitats, we used a one-way analysis of covariance (ANCOVA) with shelter habitat (water, terrestrial) as the factor and hydric state as the covariate. Individual toad hydric state was treated as a covariate in the model to control for individual differences in plasma volume differences that could have influenced corticosterone levels.

Results

TOAD OCCUPANCY AT WATER FEATURES

Our habitat occupancy surveys revealed that wherever standing water was available in the landscape, toads were present, regardless of whether habitats were natural or artificial. Toads were found at all inspected natural water holes ($n = 12$) and dams ($n = 29$) where standing water was present at ground level. Toads were not detected at dry natural water holes ($n = 15$) or troughs or steel tanks where standing water was not available at ground level ($n = 4$).

DIURNAL SHELTER SITES USED BY TOADS AROUND DAMS

We obtained 143 fixes of diurnal shelter sites from 39 individual toads. Birds killed two telemetered toads, and the transmitter fell off another toad. Toads were more likely to shelter in the water than away from the water (Cochran's Q test, $\chi^2 = 311$, d.f. = 3, $P < 0.001$). Most shelter sites were located in the water or at the water's edge (75%), and a further 9% and 5.6% of shelter sites were within 0.1–1 m and 1.1–10 m of the water, respectively (Fig. 3). All but two of the tracked toads sheltered in the water on at least one of the 4 days that tracking was conducted.

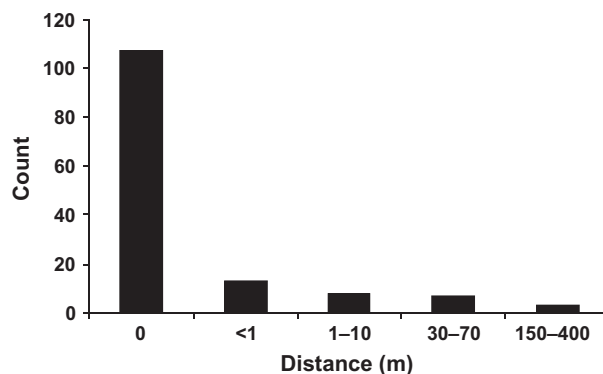


Fig. 3. The number of fixes obtained on radiotracked toads in shelter sites at different distances from water around dams. Five to seven adult toads (range: 80–120 mm SUL) were tracked at six dams ($n = 39$ total) over 4-day periods in September and October 2009. No toads were observed sheltering between 10–30 m and 70–150 m.

Although most toad shelter sites were in or immediately adjacent to the water, some toads moved considerable distances from the water (Fig. 3). Of 31 diurnal shelter sites located away from water, 19 were under dense grass clumps between 0.2 and 150 m from the water's edge, six were inside cattle hoof prints <1 m from the water's edge, two were under logs 70 m and 410 m from the water's edge, and three were in soil cracks 10–150 m from the water's edge. One toad was found buried in loose soil 10 m from the water.

WATER USE AND LONG-TERM RESIDENCY OF TOADS AT DAMS

After tracking commenced in April 2010, all 20 tracked toads were frequently detected in the water at the dams for a period of 25 days (Fig. 4). During this period, on average, toads were detected in the water 30% of the time (range: 5–66%). Toads made an average of 35 ± 4 discrete visits to the water over the 25-day period (range: 10–67 visits). Visit durations averaged 317 ± 52 min (range: 76–872), while time between visits averaged 1028 ± 301 min (range: 255–2790).

Coincident with the onset of a 10-day rainfall event (May 17–27) that delivered 44 mm of rain, 18 of the 20 toads dispersed from the dams after May 17 (Fig. 4). Of the 18 toads that left the dams, 12 did not return, while six returned and resumed residence after periods away ranging from 18 to 57 days. Six toads were detected either daily or every 2 days at the dams, indicating residence, throughout the entire 6-month dry season until tracking ceased on September 18. Four of these six toads were found alive at the water's edge at the end of the tracking period. Two toads were also detected daily or every 2 days at the dams until mid-July (4 months) and mid-August (5 months), after which they were not detected again, possibly due to mortality.

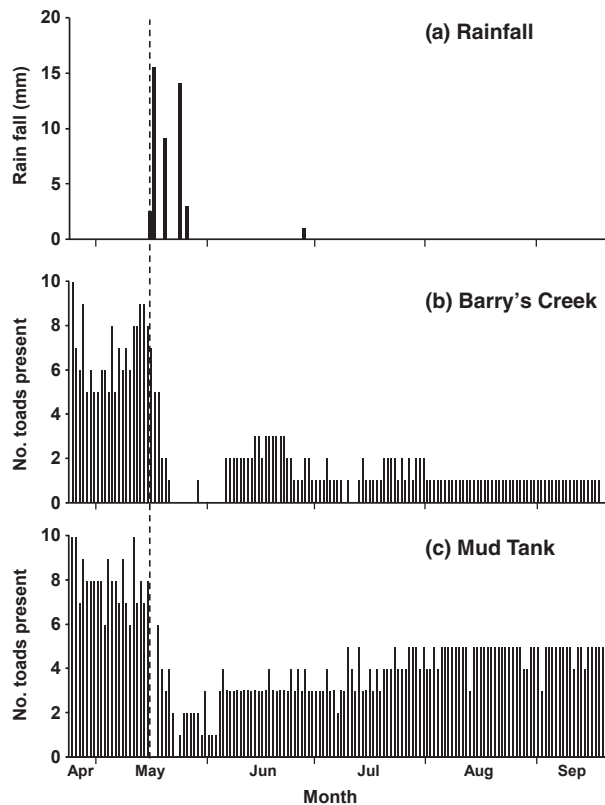


Fig. 4. (a) Daily rainfall recorded at Camfield station between April and September 2010. The number of toads with acoustic tags that were detected per day by passive acoustic listening stations at two dams, (b) Barry's Creek and (c) Mud Tank. Ten toads were tagged at each dam.

MODEL DESICCATION RATES AND TEMPERATURES INSIDE TOAD SHELTER SITES AT DAMS

Water loss of plaster models differed substantially among habitat types (ANOVA, habitat $F_{2,12} = 72$, $P < 0.0001$; dam $F_{1,12} = 4.8$, $P = 0.05$; interaction $F_{1,12} = 0.1$, $P = 0.13$). Models in the water lost little mass in comparison with models located in soil cracks and under grass, respectively (Fig. 5a).

Mean temperatures within plaster models differed between habitat types (ANOVA, $F_{2,12} = 4.8$, $P = 0.03$) and were similar across dams (ANOVA, $F_{2,12} = 0.1$, $P = 0.7$; interaction term: $F_{2,12} = 1.3$, $P = 0.31$). Mean model temperatures of models placed at the water's edge (27.2 °C, SE = 0.44) were lower than those placed in soil cracks (30.4 °C, SE = 1.16) or under grass (30.0 °C, SE = 0.52).

Maximum model temperatures varied between dams (ANOVA, $F_{1,12} = 12.3$, $P = 0.0004$), did not differ across microhabitats (ANOVA, $F_{2,12} = 1.2$, $P = 0.4$), but there was a significant interaction term ($F_{1,12} = 5.6$, $P = 0.02$). Maximum temperatures of models under grass were similar at Dry Bore (40.2 °C, SE = 1.17) and N48 (41.3 °C, SE = 1.17), but model temperatures were higher inside soil cracks at Dry Bore (41.7 °C, SE = 1.17) than at N48 (36.0 °C, SE = 3.04), as were temperatures at the water's edge (means of 43.5 °C, SE = 2.31 vs. 32.8 °C, SE = 0.33).

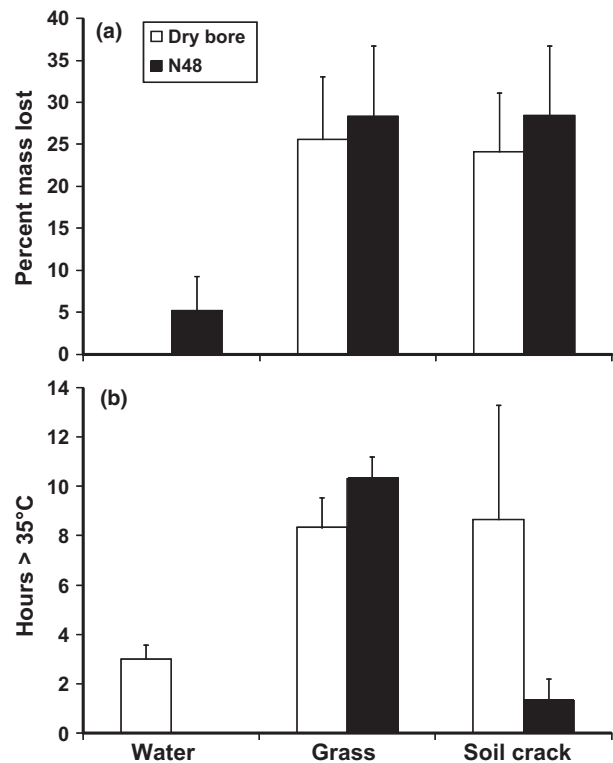


Fig. 5. Mean percentage mass loss (a) and number of hours 35 °C was exceeded (b) in plaster models deployed for 48 h in three microhabitats around two dams during two time periods. Models were placed in the field for 48 h on 13 October 2009 (open bars) and on 15 October 2009 (black bars). Error bars show +1 standard error.

The number of hours during which plaster models exceeded 35 °C over the 48-h period was influenced by both dam (ANOVA, $F_{1,12} = 4.9$, $P = 0.01$) and microhabitat (ANOVA, $F_{2,12} = 7.2$, $P = 0.001$; interaction term $F_{2,12} = 2.1$, $P = 0.06$). At both dams, plaster models under grass experienced high temperatures for >8 h during the 48-h period. Models inside soil cracks attained high temperatures for long periods at both dams, whereas models on the water's edge were buffered against high temperatures at N48, but not at Dry Bore (Fig. 5b).

PHYSIOLOGICAL STRESS IN SHELTER HABITATS

Toads that used terrestrial diurnal shelters (103 ± 10 ng mL⁻¹ plasma) to avoid the hot dry conditions of the day had 2.5 times higher corticosterone levels than toads that used the water's edge at dams (42 ± 11 ng mL⁻¹ plasma; ANCOVA, $F_{1,29} = 14$, $P = 0.001$). There was no effect of hydric state on plasma corticosterone levels (ANCOVA, $F_{1,29} = 0.1$, $P = 0.7$).

Discussion

Our results, using multiple lines of evidence, support the hypothesis that sites with permanent water function as dry-season refuges for cane toads. Cane toads were

present at all dams ($n = 29$) and natural waterholes ($n = 12$) where water was available at ground level, but were not observed at the artificial water points ($n = 4$) and dry natural waterholes ($n = 15$) where water was absent at ground level. Radiotelemetry and ultrasonic telemetry data indicate that cane toads' movements are focussed around dams during periods of dry climatic conditions because they must remain in close proximity and use them frequently to maintain water balance. Plaster models placed in toad shelter sites away from the water lost more mass and experienced higher temperatures than models placed near the water's edge. Toads that sheltered in dams exhibited less physiological stress as evidenced by lower corticosterone levels when compared to toads that resided in soil cracks. Our findings lend support to the idea that dams have facilitated the invasion of cane toads by providing toads with refuge from dehydration and extreme heat on the seasonally arid plains of our study region.

Potentially, toad presence at dry sites could have occurred if cane toads selected suitable microhabitats (e.g. deep soil cracks) which prevented desiccation, and thus, allowed them to survive the long dry season without access to standing water. However, this scenario is unlikely to occur, for several reasons. First, plaster models showed that even in deep soil cracks, cane toads lose 27–28% of their body mass over a 48-h period. Because cane toads cannot survive if they lose > 40% of their body mass (Zug & Zug 1979), toads sheltering inside soil cracks would still require regular access to standing water every 1–2 days. Secondly, all but two of the radiotracked toads sheltered in water in dams during the four-day tracking period. Further, the two toads that did not shelter in dams were sheltered within 10 m of the water. Thirdly, when water became scarce in the landscape, toads with ultrasonic trackers accessed water frequently (every 1–2 days) throughout the entire 6-month dry-season tracking period. Finally, previous studies have shown that during periods of dry conditions, cane toads are extremely susceptible to desiccation and cannot survive without access to water (Zug & Zug 1979; Reynolds & Christian 2009). Collectively, these results suggest that in the late dry season, cane toads were limited to sites where standing water was available to avoid dehydration. A similar pattern of water dependency has been documented for cane toads during the dry season in the more mesic environments of Australia's wet-dry tropics (Seebacher & Alford 2002; Brown, Kelehear & Shine 2011).

The decreased frequency of visitation to dams by toads fitted with ultrasonic tags following rainfall events during our study supports the notion that cane toads disperse between dry-season refuges following rainfall events, which occur primarily during the summer wet season. Following rainfall events, higher humidity levels and the presence of water in areas away from permanent natural waters and dams may allow toads to venture away from their refuge habitats because the risk of dehydration is

reduced (Brown, Kelehear & Shine 2011; Tingley & Shine 2011). Indeed, the mobility of toads during periods of 'wet conditions' is evidenced by studies showing that that under 'wet season' conditions, individual toads can move as far as 1 km in a 24-h period (Phillips *et al.* 2007). The likely process of invasion in our study area has been that toads have gradually expanded their range by dispersing from dry-season refuges following rainfall events in the wet season. During the dry season, water becomes a limiting resource and the distribution of toads contracts to sites with permanent water from which individuals can disperse during the next wet season. Presumably, toads that have not located permanent sources of water by the start of the dry season die from dehydration.

By providing dry-season refuges for toads, artificial water points may be expected to increase the cane toad population at a catchment-wide scale. Toad populations at the dams we surveyed were dominated by adult toads, including reproductive females, and many of the dams supported large numbers of toads. For example, during daytime surveys, we counted 819 toads at one dam and estimated that more than 500 toads were present in two other tanks we surveyed. By elevating toad densities, dams could exacerbate cane toads' ecological impacts by increasing encounter rates between toads and terrestrial predators. Thus, we predict that significant population declines of varanid lizards and snakes will occur in arid, pastoral regions where extensive networks of dams exist.

A shortcoming of our study was that we could not show unequivocally that toads would not have invaded the seasonally arid plains of our study area without the presence of dams. To demonstrate this would require comparison of habitat occupancy and the survival of toads in landscapes with low densities of natural water sources with and without dams. Such a comparison was not possible because there are no areas comparable to our study area without dams (Florance *et al.* 2011). The presence of dams is unlikely to have influenced the invasion of toads along the Victoria River and the western part of our study area owing to the relatively high density of permanent natural waterholes (Fig. 2). However, our results showing that dams function as refuge habitats for toads and those of previous studies, which have shown that toads cannot survive without access to water (Zug & Zug 1979; Reynolds & Christian 2009; Florance *et al.* 2011), suggest strongly that toads would be unable to permanently occupy the seasonally arid plains, distant from permanent natural waterholes, in the eastern part of our study area (Fig. 2) without the refuge habitats that dams provide.

If dams function as obligatory refuge habitats for toads in otherwise waterless landscapes, it follows then that there is considerable scope to control toad populations and limit their spread in arid areas by restricting their access to water. Simulation studies suggest that the spread of cane toads could be halted in landscapes where natural sources of water are scarce by systematically excluding toads from artificial water points to create waterless

barriers which individual toads cannot traverse (Florance *et al.* 2011; Tingley *et al.* 2013). At catchment scales, the implementation and success of artificial water exclusion programmes to control cane toads will be dictated by the source–sink dynamics of toad populations, in particular the density of natural water holes which are likely to function as source areas for toads and the considerable dispersal potential of toads (Florance *et al.* 2011). As toads invade progressively more arid areas of Australia, their reliance on AWP and hence the potential to control their populations by limiting their access to water should increase.

Cane toads are not the only species to benefit from the establishment of AWP in arid landscapes. The establishment of AWP has fundamentally changed once waterless arid landscapes by focusing the movements of species that drink around water sources and by facilitating the spread of native and invasive species that need to access to water to survive (James, Landsberg & Morton 1999; Krausman, Rosenstock & Cain 2006). Where invaders rely upon having access to AWP, there may be potential to manage their populations by restricting their access to water. For example, strategically placed fencing that impedes feral goats' access to AWP has been used to control the impacts of feral goats *Capra hircus* L. in arid Australia (Russell, Letnic & Fleming 2011). Such water exclusion strategies tailored for specific species could be implemented to control established populations of invaders, or conducted strategically, by rendering AWP inaccessible ahead of the invasion front to prevent further spread.

CONCLUSION

Our results show that permanent water sources serve as dry-season refuges for toads in the semi-arid study area. Dams increase the number of dry-season refuges available for cane toads and are likely to have facilitated the spread of this water-dependent invader into naturally waterless areas. We predict that dams will exacerbate the impact of cane toads in arid regions by allowing cane toads to colonize areas distant from natural water where they will kill reptilian predators that attempt to eat them. Our study suggests that preventing cane toads and other water-dependent invasive vertebrates from accessing artificial water points can be used as a strategy to control their populations in arid regions.

Acknowledgements

We thank Ben Wratten (AACo) for logistical support. The Hermon Slade Foundation (ML, TD, JKW) and Australian Research Council funded this research (JKW).

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Received 22 August 2013; accepted 27 January 2014

Handling Editor: Jonathan Rhodes

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Figures showing a livestock trough, earthen walled dam and plastic water reservoir.