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Sex and age differences in habitat use by invasive cane toads (*Rhinella marina*) and a native anuran (*Cyclorana australis*) in the Australian wet–dry tropics

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Abstract Although generalized habitat use may contribute to the success of invasive taxa, even species that are typically described as habitat generalists exhibit non-random patterns of habitat use. We measured abiotic and biotic factors in 42 plots (each 100 × 10 m) along a 4.2-km long unpaved road in tropical Australia, at a site that had been invaded by cane toads (*Rhinella marina* Bufonidae) seven years previously. We also counted anurans at night in each of these plots on 103 nights during the tropical wet season, over a five-year period, beginning soon after the initial toad invasion. Spatial distributions differed significantly among adult male toads ($n = 1047$), adult female toads ($n = 1222$), juvenile toads ($n = 342$) and native frogs (*Cyclorana australis* Hylidae, $n = 234$). Adult male toads were closely associated with water bodies used as calling and/or spawning sites, whereas adult female toads and native frogs were most commonly encountered in drier forested areas on sloping ground. Juvenile toads used the margins of the floodplain more than conspecific adults did, but the floodplain itself was rarely used. Understanding which components of the habitat are most important to specific age and sex classes within a population, or how invasive species differ from native species in this respect, can clarify issues such as the spatial and temporal location of ecological impact by an invader, and the most effective places for control of the invader with minimal collateral effects on the native biota.

Key words: *Bufo marinus*, biological invasion, generalist, habitat preference, microhabitat.

INTRODUCTION

Although some animal and plant species are found only in highly specific habitats, many others are broadly distributed across a diverse array of superficially diverse habitat types. Even for these later ‘habitat generalist’ taxa, however, detailed investigation is likely to reveal that individuals utilize some microhabitat types more often than others (Harris *et al.* 2006; Stewart *et al.* 2010; Hahn *et al.* 2011); and frequently, an animal’s usage of specific microhabitats depends upon factors such as its age, sex and reproductive state; and may shift as a function of seasonal changes in the distribution of key resources such as food, water and shelter (Bolnick *et al.* 2003; McEachern *et al.* 2006; Pandit *et al.* 2009; Browne & Paszkowski 2014). Understanding interspecific and intraspecific divergences in microhabitat use can clarify many questions in population ecology, including issues relevant to conservation and management (van Toor *et al.* 2011; Browne & Paszkowski 2014). For example, an ability

to exploit a wide range of habitat types has often been identified as a contributor towards invasion success of translocated taxa (Dukes & Mooney 1999; Darrigran 2002; Cassey *et al.* 2004; Hahn *et al.* 2011; Sol *et al.* 2012). Knowledge of which of these microhabitat types are most important, and their differential importance to age and sex classes within the population, can help to identify the most effective places for invader control (Harris *et al.* 2006). Also, quantifying microhabitat overlap between invaders and native taxa can identify which components of the local biota are most likely to be affected by an invader (Harris *et al.* 2006; Harper & Cabrera 2010).

Because of their ectothermy and water-permeable skins, amphibians depend upon environments that provide suitable hydric and thermal regimes (Jorgensen 1997; Lillywhite 2006). Hence, amphibians are discontinuously distributed across any landscape that is heterogeneous hydrically or thermally (Lillywhite 1970). In areas with seasonal precipitation regimes, anurans typically are inactive in sheltered sites within the dry season but are distributed more widely across the landscape during the wet season (Young *et al.* 2005; Tracy *et al.* 2007). An invasive

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anuran is unlikely to influence native taxa, or to be accessible to control efforts, while it is inactive in dry season refuges. Thus, the wet season is the critical time in terms of predicting the impact of an invasive species, or of identifying the best places for targeted control of an invader.

Like many invasive species, cane toads (*Rhinella marina* Bufonidae) are often described as habitat generalists; the main biases identified in published literature are the toad's preference for open rather than thickly vegetated sites and (perhaps reflecting this preference) a trend for toads to be more abundant in anthropogenically disturbed sites than in pristine habitats (e.g. Zug & Zug 1979; Lever 2001). However, data on this topic are scarce, apart from broad-scale comparisons of toad numbers in widely separated areas that differ in habitat attributes (e.g. limestone outcrops and woodland *vs.* disturbed sites: Pearson *et al.* 2009) or in areas either close to or far from specific habitat factors (e.g. lighted buildings: EG-B unpublished data). Thus, we conducted a detailed survey to quantify (i) the spatial location of male, female and juvenile toads, and of native frogs, along a 4.2-km road transect; and (ii) biotic and abiotic characteristics of sites along this transect, to compare to phenotypic traits of the anurans found at each site.

METHODS

Study site

Beatrice Hill Farm is located on the fringes of the Adelaide River floodplain, 60 km east of Darwin in the wet-dry tropics of northern Australia. The farm comprises 2600 hectares, mainly black soil floodplains (inundated for weeks to months every year after monsoonal rains) dominated by aquatic vegetation (Madsen & Shine 1999; Story *et al.* 2010) but higher and drier floodplain edges contain a more diverse flora. The most extensive ground cover consists of exotic weeds (e.g. gamba grass *Andropogon gayanus*, Calopo *Calopogonium mucunoides* and mint weed *Hyptis suaveolens*) that have been grown to provide fodder for livestock (Cook *et al.* 2005). The floodplain lacks trees, but drier ground contains extensive and floristically diverse woodland dominated by eucalypts (primarily *Eucalyptus mineata* and *E. tetradonta*: Story *et al.* 2010). The climate is hot year round (mean maximum daily air temperatures >30°C in every month) but with more than 80% of the annual precipitation falling during the relatively brief (December to March) wet season (Taylor & Tulloch 1985; Shine & Brown 2008). Activity of most native frogs begins with the first heavy rains (typically in October–November) and ends as the ground dries out (typically in May–June). Our surveys centred on a 4.2-km dirt (unpaved) road that runs through Beatrice Hill Farm (and is used only by farm personnel), traversing a variety of habitat types. For most of its length, the road runs along the edge of the floodplain, but it crosses it in some areas, and runs along higher drier ground in other sections (see Fig. 1).

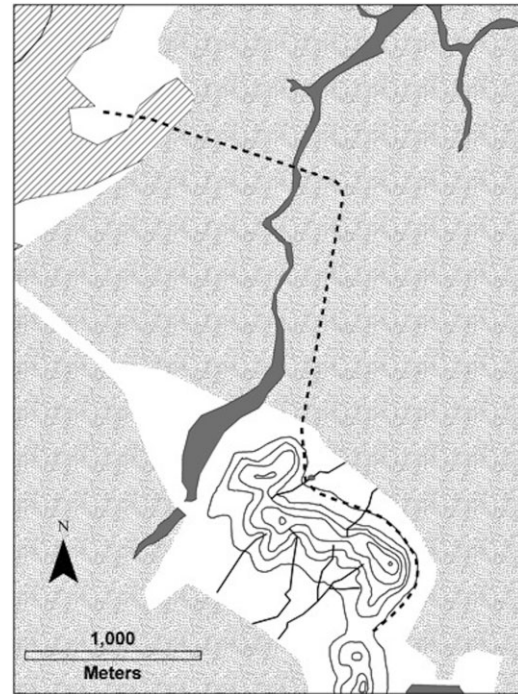


Fig. 1. Topographic map (with 10 m contour lines) of the study transect and habitat features. The dashed line indicates the surveyed roadway passing through different habitat types: floodplain (shaded), pasture (hatched) and forest (white).

Study species

The cane toad *R. marina* (*Bufo marinus* in earlier literature) is a large (up to 150 mm snout–urostyle length [= SUL]) terrestrial bufonid with a broad native range within the Americas. The toad was brought to Australia in 1935 for biological control purposes, and has since spread widely (Urban *et al.* 2008). Toads arrived in our study area in 2005 (Brown *et al.* 2006). Cane toads possess highly toxic defensive compounds, and many native predators (including fishes, frogs, lizards, snakes, crocodiles and marsupials) have been fatally poisoned when they have attempted to eat the invasive toads (Shine 2010). In our study area, cane toads remain active year-round but tend to be restricted to sites with permanent water during the dry season.

The giant burrowing frog *Cyclorana australis* (Hylidae) is a terrestrial, burrowing species found throughout the Australian wet-dry tropics. It is the largest native anuran in our study area, with an SUL up to 105 mm (males' SUL = 61–81 mm, females' SUL = 70–105 mm: Tyler & Knight 2009). These frogs are active only during the wet season; during dry periods, they aestivate in burrows and form cocoons that reduce water loss (Tracy *et al.* 2007). *Cyclorana australis* resemble cane toads in body size, body shape and general habits. Laboratory data suggest that this species behaviour is altered by the presence of cane toads, for example, enclosure trials suggest that these frogs become less active in the presence of cane toads (Greenlees *et al.* 2007), and that metamorph *C. australis* avoid shelter sites that contain scent cues from cane toads (Pizzatto & Shine 2009). With these precedents, it is important to find possible effects of cane

toads on this species in the field. Although we saw other species of frogs on our surveys, these were smaller, more mobile and difficult to identify with certainty. Thus, our analysis of native anurans is based only on *C. australis*.

Field surveys

Surveys were conducted between 2000 h and 2200 h from a slow-moving (10–15 km/h) all-terrain vehicle that traversed the entire 4.2 km length of the road. Counts were conducted on outbound trips (north to south) only. We recorded locations of each toad and *C. australis* on the road surface using a handheld GPS unit. In the case of toads, we also recorded the sex of each adult individual (females grow larger, have less rugose skin and are brown rather than yellow: Zug & Zug 1979). Toads >90 mm SUL were scored as adults; smaller toads were scored as juveniles, and we did not attempt to determine their sex. A total of 103 surveys were conducted during wet season months (approximately November–May), when amphibian activity is highest, beginning in April 2006 and ending in April 2010. Nine surveys were carried out in the 2005–2006 wet season, 17 in 2006–2007, 43 in 2007–2008, 23 in 2008–2009 and 11 in 2009–2010.

Characterization of habitats

For analysis of our data, we divided the road into 42 sections, each 100 m long. We divided each of these 100-m lengths into two survey plots, one on either side of the road (10 m wide beginning at the edge of the road). Thus, a total of 84 plots were evaluated. In each of these plots, we recorded ground cover in terms of the percentage of open soil, leaves, grass, plant, shrubs and tree coverage. At the beginning of each plot, we also measured the slope using a clinometer (Suunto PM-5/360 PC). To quantify other traits more accurately, we marked points every 33 m, within the 10-m width but at randomly selected distances from the road. At each of those points we measured leaf litter depth, grass depth and soil softness. Soil softness was estimated as the depth (cm) that we were able to penetrate with a garden shovel applying a (subjectively) standardized force. All habitat data were scored by the same person (EG-B).

We also took soil samples ($n = 252$), and returned these to the laboratory for measurement of water retention capacity. Each sample was dried at room temperature until total mass remained unchanged for three days. Three subsamples, 10 mL in volume, were taken from each of the 252 original samples. Each subsample was then weighed and placed on a filter paper cone (Whatman 2.90 mm) set on a plastic tube; 10 mL of water was then added. Forty minutes later, we weighed the sample and calculated the proportion of water it retained relative to its original dry mass. The average water retention value for the three subsamples per plot was used for analysis.

Canopy openness was determined from pictures taken with a Nikon Coolpix 995 digital camera with a Nikon FC-E8 fisheye converter lens at each of these points. Each picture was taken placing the camera on a flat surface (0°) and oriented to the magnetic north. Three pictures at three different points were taken for each transect. Each picture

was then analysed using the program Gap Light Analyzer version 2.0 (www.ecostudies.org/gla/). Prior to analysing the photos, site-specific information on locality (latitude/longitude) and day-length were obtained from <http://www.ga.gov.au/> and averaged monthly from November to April. Gap Light Analyzer uses this information to quantify canopy cover expressed as the percentage openness.

Statistical analysis

After dividing distances along the road into 42 sequential 100-m bins, we used contingency table chi square tests to look for differences in the spatial distributions of (i) male *versus* female adult toads, (ii) adult *versus* juvenile toads and (iii) toads *versus* native frogs (*C. australis*).

We then used Canonical Correspondence Analysis (hereafter CCA) (Shanmuganathan *et al.* 2010) to identify environmental factors that predict the spatial distribution of subgroups (juveniles, adult males, adult females) within the cane toad population and of *C. australis*. CCA detects the patterns of variation in taxon occurrence that are best explained by linear combinations of environmental variables (Ter Braak 1986). CCA generates an ordination diagram in which focal taxa are represented by points and environmental variables are represented by arrows. The directions of the arrows represent maximum change of that environmental variable, and longer arrows represent variables with greater rates of change. If the central point for a taxon lies on an arrow, it means that that species (or sex, etc.) is found most often at sites that have a specific value for the environmental characteristic represented by that arrow. We used the total number of organisms (e.g. male toads) observed on each plot as dependent variables. We excluded six highly correlated ($r > 0.7$) variables relating to depth of the leaf litter, percentage of grass coverage, grass layer depth, shade coverage, percentage of shrub coverage and number of trees, leaving a set of 14 relatively uncorrelated environmental variables. All analyses were done in the statistical package ‘vegan’ (Oksanen *et al.* 2011).

A Mantel test based on Spearman’s rank correlation (ρ) with 999 permutations was performed to evaluate the capacity of the environmental variables included in the CCA to predict the distribution of cane toads (separately by sexes and adult *vs.* juvenile) and *C. australis*. The Mantel test compares matrices of correlations to determine if there is a statistical association between corresponding elements.

We also examined another variable: the distance from each plot to the closest breeding pond (based on calling by male toads and/or the presence of eggs and tadpoles). This variable was not included in the CCA but might be important as a measure of proximity to breeding sites. We used linear regressions to evaluate relationships between the total numbers of anurans (separately for female, male, juvenile cane toads and *C. australis*) and this distance measure. We also examined shifts in toad population structure and sex ratio using ANCOVA, with distance to the breeding pond and number of toads (or adult toads only) as covariates and numbers of juvenile toads (or adult male toads) as dependent variables. Using the same method, we examined numbers of *C. australis* relative to all anurans as a function of distance from the nearest pond.

RESULTS

We recorded a total of 2611 cane toads (1222 females, 1047 males and 342 juveniles) and 234 *C. australis* along the road during the survey period. The road crosses a range of habitat types, beginning from the south with a sloping (maximum of 12° slope) densely wooded section at 17 m above sea level (a.s.l.) among hills, down through lower areas with fewer trees and abundant shrubs, and then further down into frequently inundated flat areas (4 m a.s.l., 0° slope; see Fig. 2). This later (floodplain) habitat has very few trees but a thick cover of grasses (*Hymenachne* sp.) and contains soil that was able to retain water for longer (indicating high clay content). The road then runs along the edge of the floodplain into grass-covered paddocks used for livestock (water buffalo) production. Within the 10-m wide plots that we scored along this 4.2 km length of road, 14% of the ground surface was covered by gamba grass

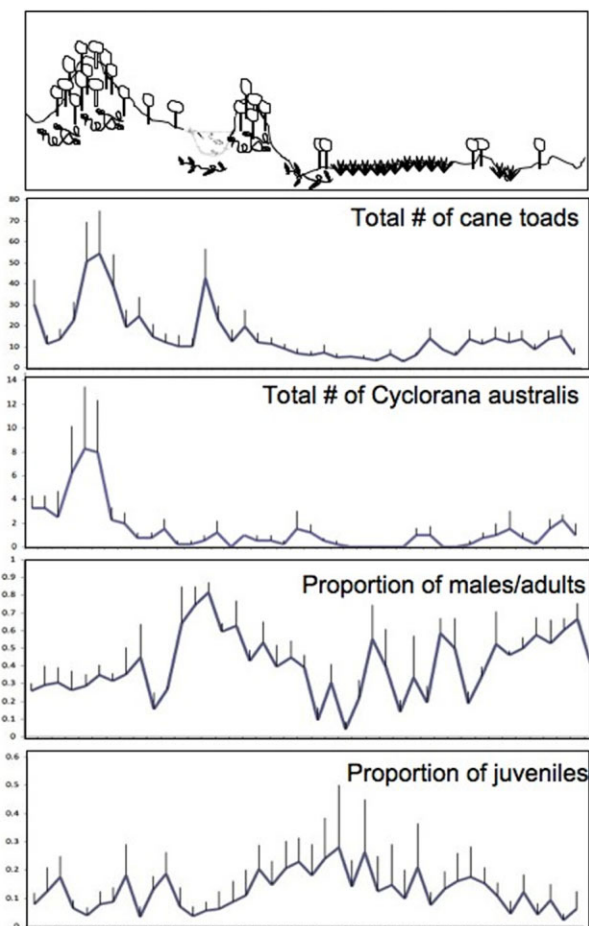


Fig. 2. Spatial distribution of habitat features found along a 4.2 km road, and the distribution (mean and SE) of cane toads (*Rhinella marina*), native frogs (*Cyclorana australis*) and proportions of male and juvenile cane toads along the road.

(*Andropogon gayanus*), 24% was covered by floodplain grass (*Hymenachne* sp.), and 7% was covered by *Calopogonium* sp. (a vigorous, creeping herb that grows several metres long and forms a tangled mass of foliage 30–50 cm deep). *Hyptis suaveolens* (a perennial herb that can grow up to 1.5 m, and is commonly found alongside roads, watercourses and overgrazed pasture) was present on 4% of the total area. Trees occurred along only 8% of the road's length, and were concentrated in higher areas with steeper slopes. Due to the presence of trees, this 8% also had higher leaf litter cover and a less open canopy (canopy cover at ground level varied from 60% to 97% overall). Introduced lawn grass covered 16% of the plots along the sides of the road, with another 7% covered by shrubs and other plants. Bare soil was present on 16% of the area, and leaf litter on 4%. The water retention capacity of soil changed along the length of the road, with higher values in the low-lying floodplain sections.

Chi square analysis showed that the distribution of male toads along the road differed from that of females ($\chi^2_{(1,41)} = 284.18$, $P < 0.0001$), the distribution of juvenile toads differed from that of adults ($\chi^2_{(1,41)} = 156.59$, $P < 0.0001$), and the native anuran *C. australis* was distributed differently to toads ($\chi^2_{(1,41)} = 140.8$, $P < 0.0001$; see Fig. 2).

The numbers of juvenile toads, relative to adults, also differed among years; during 2006 the proportion of juveniles was around 50%, whereas during the other years it was less than 20% ($\chi^2_{(4)} = 221.706$, $P < 0.0001$). Among adult toads, the proportion of males to females was virtually 1:1, except for 2009 when the proportion of males was only 40% ($\chi^2_{(4)} = 29.835$, $P < 0.0001$). The numbers of *C. australis* relative to the total number of cane toads was low in 2006 (when the native species comprised only 1% of the sightings), whereas during the remaining years it was approximately 5% ($\chi^2_{(4)} = 17.035$, $P < 0.001$).

The two axes of the CCA ordination depict patterns of variation among sexes and age-classes of cane toads, and individuals of *C. australis*, relative to the habitat variables that we measured. The perpendicular intersection of a vector with a point (e.g. for male toads) represents the approximate centre of the organism's distribution along the environmental gradient represented by that vector. Habitat preferences of the native frog *C. australis* were similar to those of adult female cane toads (Fig. 3). Individuals of both of these groups were most likely to be encountered in plots with higher slopes, more trees, a thick understory (mostly due to the presence of *Calopogonium*) and a higher percentage ground cover of soil and leaves. The position of female toads and *C. australis* within the CCA diagram, relative to the vectors representing soil water retention, canopy openness and floodplain grass, suggests that both of these anuran groups avoid the floodplain, and prefer sites with more canopy coverage and soil that is

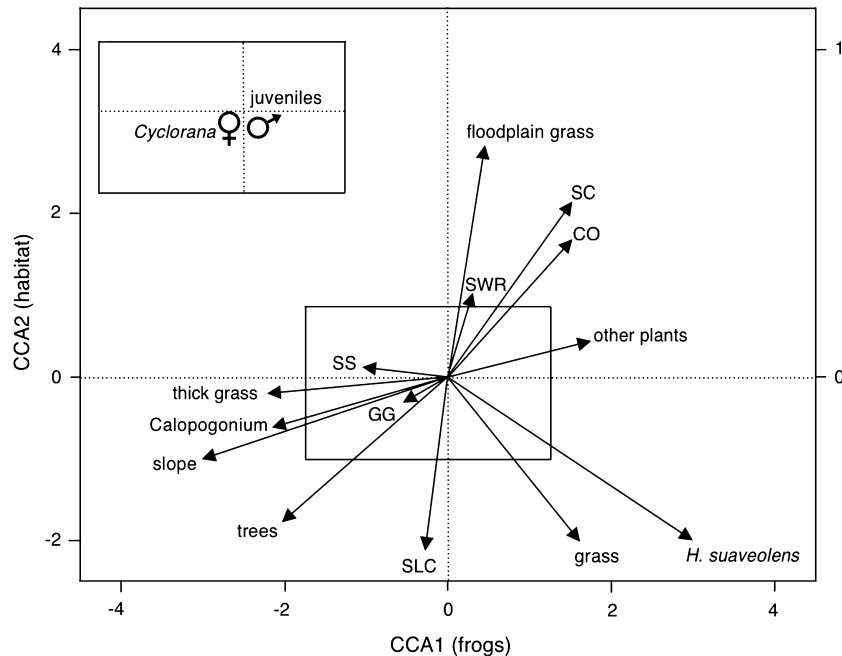


Fig. 3. First two axes from the canonical correspondence analysis of the distributions of environmental variables and of cane toads (separately by sexes and ages) and the native frog *Cyclorana australis* (all sexes and ages combined). Arrows represent environmental variables while the location of the organisms in reference to them is shown in an expanded rectangle (♀, ♂ and juvenile cane toads and *C. australis*). SS = soil softness, SWR = soil water retention, SC = soil coverage, SLC = soil and leaves coverage, CO = canopy openness, GG = gamba grass.

unlikely to become waterlogged (i.e. retains less water). In contrast, juvenile cane toads were found most often in open sites where the soil retains more water. Although that preference resulted in juvenile toads being more closely associated with the floodplain than were adult conspecifics (or native frogs), the CCA diagram shows that the central point for juvenile toads is located close to the base of this vector (Fig. 3). That is, juvenile toads tended to be on the margins of the floodplain, not deep within it where vector values were more extreme. The distribution of adult male toads was most highly associated with the presence of the herb *H. suaveolens* and with the presence of lawn grass (Fig. 3).

The Mantel test showed that the habitat variables that we measured were significantly correlated with the distribution of anurans ($r = 0.21$, $P = 0.01$). That is, areas with similar environmental conditions contained similar numbers and types of anurans.

Our linear regressions showed that male cane toads were encountered more frequently closer to breeding ponds ($r^2 = 0.19$, $F_{(1,41)} = 9.64$, $P = 0.003$), whereas females ($r^2 = 0.003$, $F_{(1,41)} = 0.12$, $P = 0.72$) and juveniles ($r^2 = 0.007$, $F_{(1,41)} = 0.29$, $P = 0.58$) were not. Distance to ponds was similarly unimportant for native frogs (total numbers of *C. australis*, $r^2 = 0.014$, $F_{(1,41)} = 0.60$, $P = 0.44$). Restricting analysis to adult cane toads, the proportion of males (as well as the

absolute numbers of males) was higher closer to breeding ponds ($r^2 = 0.3$, $F_{(1,41)} = 17.83$, $P = 0.0001$). In contrast, the proportion of juveniles increased with distance to the breeding ponds ($r^2 = 0.1$, $F_{(1,41)} = 5.04$, $P = 0.03$). Distance to breeding ponds did not significantly affect the numbers of *C. australis* relative to the total number of anurans ($r^2 = 0.009$, $F_{(1,41)} = 0.39$, $P = 0.53$). To circumvent statistical problems associated with ratio measures, we repeated these analyses using ANCOVA. The number of adult male toads (dependent variable) relative to the total number of adult toads (covariate) was significantly affected by distance to the nearest pond (interaction $F_{(3,41)} = 132.60$, $P < 0.0001$). The same was true of the number of *C. australis* relative to the total number of anurans (interaction distance to pond*anuran number $F_{(3,41)} = 25.57$, $P < 0.0001$) but not for juvenile toads relative to the total number of toads (interaction number of toads*distance to pond $F_{(3,41)} = 0.71$, $P = 0.71$).

DISCUSSION

Use of disturbed sites is often characteristic of 'habitat generalist' species, and also of invasive species (Hahn *et al.* 2011). A more detailed understanding of habitat use by 'generalist' invasive fauna could lead to more

effective management strategies as well as a better understanding of invader impacts. Contrary to the idea that cane toads are 'habitat generalists', we found distinct habitat associations. Species with broad habitat use are predicted to be better invaders (Townsend 1996; Sakai *et al.* 2001; Tuomainen & Candolin 2011), but the evidence is equivocal: ecological widths of invasive species do not always differ from those of native species (Rehage *et al.* 2005). A preference for disturbed habitats may also increase the probability of a species being translocated from its original range (Van Kleunen *et al.* 2010). In our study, a 'generalist' invasive species used certain habitat types more than others, and specific sex and age groups differed in the habitats they selected. Fine-scale differences in habitat use may be most evident during periods of peak activity when resources are most widely available, and habitat options are greater.

Our detailed analysis revealed highly non-random associations between toad abundances (as inferred from encounter rates during nocturnal surveys) and a suite of abiotic and biotic characteristics of the environment. Importantly, each of the cane toad groups that we investigated (adult males, adult females, juveniles) differed from each other in their locations (and thus, in the habitat characteristics that predicted their occurrence). The only two groups that preferred habitats with similar habitat characteristics were female toads and native frogs as revealed in the CCA analysis.

Inevitably, there are caveats to our analysis. Our measures of anuran location were based on visual encounters, so that they may not relate directly to underlying abundances. For example, anurans might be seen more easily in some habitats than in others, or our counts may be inflated by repeated sampling of the same individuals. Neither of these biases is likely to affect our main conclusions, however. First, the relatively open terrain (especially, the surface of the road), coupled with a tendency for toads to select very open microhabitats at night (Lever 2001; Brown *et al.* 2006), reduces any observability biases. Second, although we undoubtedly saw the same individuals more than once, the large sample size and long duration of sampling (five years) minimizes any impact of this pseudoreplication. At this site, mark-recapture and radiotelemetry studies on toads have shown that during the wet season (when our surveys were done) most adult individuals are transitory rather than philopatric (Brown *et al.* 2011). Thus, methodological issues should not compromise the validity of our major conclusions about habitat use.

Another caveat is that our surveys were conducted only at one time of year: the wet season. For most of the rest of the year, virtually all native frogs and many toads are inactive, and often sequestered deep inside

burrows or under other forms of shelter (Greenlees *et al.* 2007; McArthur 2007; Tracy *et al.* 2007). Inevitably, those shelter sites are discontinuously distributed across the landscape, because opportunities for rehydration are scarce in the semi-arid conditions that are experienced throughout this period (Young *et al.* 2005). Thus, demonstrating non-random habitat use by anurans during the dry season would not be of great biological interest: spatial heterogeneity in water availability would inevitably produce such heterogeneity in anuran distributions. This constraint is largely or entirely removed during the wet season, a time when anurans can readily maintain their hydric balance across most of the landscape. Finding strong microhabitat selection at this time of the year (as our results demonstrate) cannot be attributed simply to hydric constraints. Even when water is freely available across the landscape, the anuran taxa that we studied showed highly non-random use of habitats (and indeed, avoided the wettest sites).

Why are different anuran species, and different age and sex classes within at least one of those species, distributed in different ways across the landscape? Our data reveal biases at two levels. First, some readily available habitats were rarely used by anurans of any kind. Second, within the range of habitats that were used, some species (or subgroups within a species) exhibited differential use of specific microhabitats. In terms of the first bias, a very strong pattern is evident: we saw far more anurans (of both species) on higher, drier, forested areas at the fringe of the floodplain, than on the floodplain itself (Fig. 2). At first sight, this pattern is perplexing: the floodplain provides abundant water, thick vegetation and many insect preys. However, it also contains abundant predators (such as native rats *Rattus colletti*; Cabrera-Guzman *et al.* 2014). In addition, the floodplain can be completely inundated during the wet season, leaving little dry land for toads to shelter on. Water buffalo traversing the mud create deep pits, making it difficult for toads to travel over. In the case of *C. australis*, floodplain soils with high clay content may become so hard that frogs are unable to dig out of it at the end of the dry season. Hence, *C. australis* may avoid such areas at the end of the wet season, when they select sites in which to dig their burrows and form cocoons to spend the dry season (Tracy *et al.* 2007). On the other hand, the lush vegetation on higher areas during the wet season provides abundant shelter, favourable hydric and thermal conditions and access to terrestrial insects.

Even within the higher drier areas preferred by anurans, there were significant differences among groups in actual distributions (Fig. 2). What advantages and disadvantages might drive such divergences? The tendency of juvenile toads to be most commonly found in areas surrounding the breeding ponds may

reflect relatively slow dispersal away from natal sites (Child *et al.* 2008). The concentration of adult toads around spawning ponds clearly reflects reproductive activity: male bufonids often spend long periods calling for mates from pondside locations, inevitably skewing the sex ratio at such sites (Davies & Halliday 1978; Wells 2007). In contrast, female bufonids visit breeding ponds only to oviposit. The preference of adult female toads and *C. australis* for drier, more densely forested areas is less easily explained, but these areas may provide sites where predation rates are lower (because of escape from the floodplain-restricted predators) and where food supply and refuge sites are abundant due to the leaf litter generated by trees and a dense layer of understory vegetation (especially, *Calopogonium*). Future work could usefully compare insect abundance in the microhabitats used *versus* ignored by toads.

The divergence in habitat use within the cane toad population, as a function of an individual's sex and body size, has several implications. First, it may affect the ecological impact of toads. Many of the native predators most at risk from lethal toxic ingestion of toads are gape limited, and thus are vulnerable only to toads that are small enough to ingest (Shine 2010). Hence, small predators such as frogs, small dasyurid marsupials, snakes and bluetongue skinks may be most at risk in the places where juvenile toads congregate: that is, on the fringe of the floodplain, in microhabitats intermediate between breeding ponds and higher drier areas. In contrast, larger predators that attack adult toads (such as quolls or large varanid lizards) will be imperilled primarily around breeding ponds (where they will encounter adult male toads) or in drier forested areas (where they will encounter adult female toads).

Survey data and outdoor-enclosure experiments suggest that cane toads have relatively little effect on native frogs but may induce subtle behavioural changes (Greenlees *et al.* 2007; Semeniuk *et al.* 2007). If native frogs avoid retreat sites occupied by cane toads (perhaps based on scent cues: Pizzatto & Shine 2009) or reduce their activity levels in relation to cane toad densities (Greenlees *et al.* 2007), then habitat overlap with toads may determine the magnitude of any such effects. Based on our results, there is high overlap between the areas used by adult female toads and at least one native frog species (*C. australis*).

Within-population heterogeneity in habitat use also affects the vulnerability of cane toad populations to the control efforts often deployed against this invasive species. 'Toad-busting' efforts by local community groups and wildlife management authorities typically target sites where the largest numbers of toads can be collected; and often, these are the areas around spawning ponds, where many adult male toads aggregate to call. As a result, collections of cane toads made around

ponds are likely to be heavily biased towards adult males. Because female toads only spawn once or twice a year, whereas males are reproductively active for several months, a decrease in the number of adult males is unlikely to translate into a decrease in total reproductive output by the toad population. Given the large numbers of toads, their wide dispersion across the landscape, and their ability to make long-distance dispersal movements (Brown *et al.* 2006) even vigorous culling is unlikely to remove all of the adult male toads within a population. Hence, an adult female moving to a pond to spawn is likely to have little difficulty in locating a willing mate. A clear implication of these results is that 'toad-busts' should focus on the sexes rather than simply numbers of toads that are collected; and that searches in drier forested areas, although yielding fewer toads, might actually be more effective in reducing toad densities than pond-focused searches.

Lastly, the associations between anurans and habitat features may help us to predict the impacts of future shifts in habitat, driven by climate change or anthropogenic disturbance. For example, livestock grazing in the Northern Territory may be promoting woody vegetation (Sharp & Whittaker 2003); but the spread of pasture grasses may increase fuel loads and thus, transform some of the existing high-elevation forest areas into grassland monocultures (Phil & Andrew 2008). Either of these outcomes would affect habitat suitability differently not only for different species of anurans but also for different age and sex classes within the cane toad population. Changing the relative proportions of habitats preferred by male, female or juvenile toads might well influence future trends in toad population structure and density. A detailed knowledge of microhabitat use by invasive species may reveal preferences that could help us to understand the invader's impact and suggest opportunities for control. More generally, detailed analysis of habitat use should precede investment into management (whether it be for invader control or conservation of an endangered taxon), in order to ensure that the time and effort devoted to management is deployed in the most effective ways and in the most effective places.

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