Competing tadpoles: Australian native frogs affect invasive cane toads (*Rhinella marina*) in natural waterbodies

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Abstract   The cane toad (*Rhinella marina*) is one of the most successful invasive species worldwide, and has caused significant negative impacts on Australian fauna. Experimental work in the laboratory and in mesocosms has shown that tadpoles of native frogs can affect survival, size at metamorphosis and duration of larval period of cane toad tadpoles. To test if these effects occur in nature, we conducted a field experiment using two temporary ponds where we set up enclosures with tadpoles of native green tree frogs (*Litoria caerulea*) and cane toads in treatments with a range of densities and combinations. The presence of green tree frog tadpoles significantly decreased the growth rate of toad tadpoles and increased the duration of their larval period in both ponds; in one pond, frog tadpoles also significantly reduced the body length and mass of metamorph toads. Toad tadpoles did not have any significant negative effects on green tree frog tadpoles, but there was strong intraspecific competition within the latter species: increased frog tadpole density resulted in increased larval period and reduced survival, growth rate and size at metamorphosis for frogs at one or both ponds. Our results are encouraging for the possibility of using native frogs as one component of an integrated approach to the biological control of cane toads.

Key words: alien species, anuran, biotic resistance, *Bufo marinus*, competition, larvae.

INTRODUCTION

Understanding the mechanisms by which some invasive species succeed in a new environment, while others do not, is an important challenge for wildlife biologists. Interactions between an invader and native species can influence the success of an invasive species in a new area. The failure of many introductions may be due to biotic resistance (Elton 1958; Levine *et al.* 2004) and conversely, invasion success can be facilitated by an invader's competitive superiority over native species (Mooney & Cleland 2001). In cases where native fauna can slow or halt the invader's spread, we may be able to intensify such effects to achieve better control of an unwanted pest (e.g. Ward-Fear *et al.* 2010a).

Cane toads (*Rhinella marina* Bufonidae) are large toxic anurans that were introduced to Australia in 1935 (Lever 2001; Kraus 2009). Since their arrival, the toads have spread across a broad area of tropical and subtropical Australia, where they have encountered a diverse array of native species that can potentially act as competitors and/or predators. The toad's defensive toxins have proven fatal to many anuran-eating native predators (including snakes, lizards, crocodiles and marsupials: Shine 2010). That severe ecological impact has stimulated intensive research on ways that we might reduce toad numbers. One promising area involves the discovery that toads are vulnerable to predation by several taxa of native invertebrates, including ants (Ward-Fear *et al.* 2009) and aquatic coleopterans and hemipterans (Cabrera-Guzmán *et al.* 2012). Also, the tadpoles of cane toads co-occur with those of native anurans (Crossland *et al.* 2008), raising the possibility that competition between toad tadpoles and frog tadpoles might suppress toad recruitment.

In keeping with this possibility, laboratory and small-scale artificial pond experiments have demonstrated that not only can cane toad tadpoles negatively affect the growth and survival of tadpoles of native frog species (e.g. Williamson 1999), but the reverse is true also. That is, the viability of cane toad tadpoles can be reduced by the presence of native frog tadpoles (Alford 1999; Crossland *et al.* 2009; Cabrera-Guzmán *et al.* 2011). Recently, Cabrera-Guzmán *et al.* (2011) demonstrated in the laboratory that tadpoles of native green tree frogs (*Litoria caerulea* Hylidae) have particularly strong negative effects on the viability of cane toad larvae, reducing the toads' survival rate and size at metamorphosis (length and mass) and increasing the duration of their larval period.

Although these results suggest that frog larvae may suppress toad larvae in the field, the extrapolation is not straightforward. Concurrent mesocosm and natural waterbody experiments sometimes yield very different results, and some of the processes detected under laboratory conditions are not seen when the work is repeated in nature (Skelly & Kiesecker 2001; Melvin &

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Houlahan 2012). Thus, we need field trials (*sensu* Schoener 1983) to determine whether or not competition occurs between the tadpoles of green tree frogs and cane toads in natural ponds. Knowing whether or not trials in such waterbodies support the laboratory studies is a critical step before we can proceed to trial the idea of using frog tadpoles as part of a broad-based approach to reduce cane toad recruitment.

METHODS

Green tree frogs (*Litoria caerulea*)

This species is broadly distributed from western and northern Australia to central coastal New South Wales. Breeding takes place in the wet season from November to March in shallow, temporary waterbodies such as roadside ditches, flooded grassland, ponds, swamps and water troughs on farms (Anstis 2002; Tyler & Knight 2009; M. R. Crossland, pers. obs., 2009). The eggs (about 2000) are laid in clumps of several hundred. Tadpoles are omnivorous, feeding in any area of the waterbody (Anstis 2002), and complete their development in about 6 weeks under natural conditions (Tyler & Knight 2009; M. R. Crossland, pers. obs., 2009). Tadpoles of green tree frogs co-occur with cane toad tadpoles in breeding ponds across the Australian tropics (M. R. Crossland, pers. obs., 2010); the frog tadpoles do not prey on live toad tadpoles, but readily consume toad eggs and, if they do, are killed by the toxins therein (Crossland & Shine 2010).

Cane toads (*Rhinella marina*)

This species is native to Mexico, Central and South America, and was introduced to north-eastern Australia as a biological control agent of insect pests of sugarcane (Lever 2001). Cane toads have now spread throughout much of Queensland, northern NSW, the wet–dry tropics of the Northern Territory (NT) and northern Western Australia (Phillips *et al.* 2007; Shine 2010). Females lay eggs (up to 30 000 eggs per clutch – Zug & Zug 1979) in long, continuous strings of jelly often around vegetation in shallow pools with open (unvegetated) gradually sloping muddy banks (Anstis 2002; Hagman & Shine 2006; Tyler & Knight 2009). Cane toad tadpoles are bottom dwellers and exhibit schooling behaviour (Anstis 2002; Tyler & Knight 2009). Individuals feed on algae but are also voracious predators of conspecific eggs (Lever 2001; Crossland & Shine 2011). All stages in the cane toad life cycle contain bufadienolides (the cardioactive steroids believed to be the principal cane toad toxins), but toxin type and quantity (and thus, effect on predators) vary through toad ontogeny (Hayes *et al.* 2009).

Study area

We worked at two seasonally inundated ponds in the Adelaide River floodplain, 60 km east of Darwin, NT, in the Australian wet–dry tropics. The ponds that form every year (during the wet season) at these locations are used as breeding sites both by native frogs and by cane toads. Pond 1 is located near Middle Point village (12°34′54″S, 131°18′51″E). This waterbody (63 m long × 16 m wide, 1.5 m maximum depth) is characterized by sandy soil, clear water, and an absence of aquatic vegetation and algal cover on stones and other submerged surfaces, reflecting low primary productivity. The pond is surrounded by native trees and contains abundant coarse woody debris and leaf litter. Pond 2 is an artificial dam on Beatrice Hill Farm (12°38′24.6″S, 131°18′56.5″E), 8-km distance from Pond 1. This pond is similar in overall size to Pond 1 (45 m length × 34 m width, 1.5 m maximum depth) but has muddy soil, dark water and profuse aquatic vegetation with extensive algal growth (reflecting high productivity due to nutrients washed in from faeces of cattle and buffaloes that graze on the surrounding pasture). There are fewer trees surrounding this dam so the coarse woody debris and leaf litter intake are lower than in Pond 1. In April 2010 (11 months before performing the experiment), we erected an electric fence to exclude cattle and buffaloes from this pond.

Experimental enclosures and animals

We constructed 50 rectangular enclosures, each consisting of a PVC frame (polyvinyl chloride tubes of 20-mm diameter) and a flyscreen mesh cage (mesh 1 mm, Cyclone Industries; 92-cm length, 60-cm height, 40-cm width). The cages were designed to be submerged in a waterbody, in contact with the substrate. Mesh on the base of the cage ensured that tadpoles did not escape. The top of each cage was equipped with a zipper to exclude predators and to allow us to open and close the cages to collect metamorph anurans. A floating square of styrofoam inside each cage allowed metamorphs to emerge from the water.

Three egg clutches of *Litoria caerulea* were collected in a natural temporary pond at the study site on 13 February 2011, and three egg clutches of cane toads were obtained on March 19 and 20 by inducing spawning of adult toads collected in the same area (by injecting 0.25 mg mL^{-1} leuprorelin acetate, Lucrin, Abbott Australia). Prior to use in the experiment, tadpoles of both species were maintained as separate clutches in 750 L mesocosms containing 600 L of non-chlorinated bore water set in a shaded outdoor area, and were fed algae pellets and frozen lettuce. Water was changed twice weekly.

Experimental protocol

The field experiment was performed between March and May 2011. We collected organic matter (leaf litter and sticks, mainly from *Eucalyptus* spp.) around the ponds and dried it in the sun for 20 days, after which we added 600 g of dry matter to every experimental mesh enclosure. To allow the floating organic matter to settle to the bottom of the enclosures and develop a biofilm, the cages were placed in the ponds 13 days before the start of the experiment. Tadpoles thus could obtain nutrition from organic matter, mesh biofilm and any suspended material that entered cages through the mesh. We set up five randomized blocks of cages in each pond, with each block containing a replicate of each treatment (thus, $n = 5$

replicate cages per treatment). The treatments consisted of: (i) 30 *R. marina*; (ii) 30 *R. marina* plus 30 *L. caerulea*; (iii) 30 *L. caerulea*; (iv) 60 *L. caerulea* plus 30 *R. marina*; and (v) 60 *L. caerulea*, for a total of 450 individuals of *R. marina* and 900 of *L. caerulea* used in each pond. This design allowed us to assess the effect of increasing *L. caerulea* density on *R. marina* performance (treatments i, ii, iv), while also assessing the 'cost' of interspecific *versus* intraspecific competition for *L. caerulea* (treatments ii, iii, iv, v). We did not assess intraspecific density effects of *R. marina* tadpoles because these relationships have been quantified previously (Hearnden 1991; Alford 1994). The number of *R. marina* tadpoles used in the treatments was based on published densities in Australian waterbodies (Alford *et al.* 1995; mean density ranging from 0.11 to 0.45 tadpoles per litre). Tadpoles of *L. caerulea* also often occur at high densities (E. Cabrera-Guzmán & M. R. Crossland, pers. obs., 2010; M. Anstis, pers. comm., 2012); the densities used in this experiment were based on our unpublished observations (2009, 2010).

We randomly selected tadpoles from the different clutches, such that every cane toad clutch contributed 10 individuals to each cage containing toads ($n = 30$ tadpoles per cage), and each tree frog clutch contributed 10 or 20 individuals to each cage including the native species ($n = 30$ or 60 tadpoles per cage). We also randomly selected and measured 10 tadpoles per treatment for each pond at the start of the experiment. *Litoria caerulea* tadpoles were larger and at a more advanced developmental stage (mean snout–vent length (SVL) 12.63 mm, mean Gosner (1960) stage 32) than were toad tadpoles (mean SVL 4.11 mm, mean stage 26), to mimic size and developmental differences associated with natural phenology in the study site (green tree frog tadpoles attain a maximum body size up to three times that of cane toad tadpoles, and green tree frog adults often breed earlier than cane toads; M. R. Crossland, pers. obs., 2010).

Tadpoles were added to the cages on 23 March 2011 in Pond 1 and 1 day later in Pond 2. We deployed thermochron temperature loggers (iButton: Maxim Integrated Products Inc., Sunnyvale, CA) inside balloons suspended 20 cm below the water surface in the central cage of each block; these recorded temperature at 1-h intervals. We submerged each cage such that water depth was 54 cm (approximate volume 200 L). We checked the level of the water every day and moved cages to deeper or shallower areas as water levels in the ponds changed.

Cages were checked daily, and tadpoles with forelimbs present were collected, and kept in 1-L rectangular plastic containers until metamorphosis was completed (defined as complete resorption of tail). For each metamorph, we recorded time to metamorphosis (as number of days since the animal was added to the pond), snout–urostyle length (SUL) and mass (blotted dry weight: A&D Company Limited digital balance, Model FX-200*i*WP).

Statistical analyses

Our analyses were based on mean values per experimental cage to avoid pseudoreplication. Survival data were arcsine-square root transformed prior to analysis; all other responses were log transformed ($\ln n + 1$) to meet assumptions of normality and homogeneity of variances. The proportion of animals surviving to metamorphosis, duration of larval period, body size at metamorphosis (length and mass) and the growth rate (calculated as (metamorph SUL – initial SUL) / number of days of larval period) for each species were compared among treatments using MANOVA.

For responses of cane toad larvae, the factors included in the MANOVA were: number of green tree frog tadpoles, site and the interaction between number of green tree frog tadpoles*site. For responses of green tree frog larvae, the MANOVA included: number of green tree frog tadpoles, number of cane toad tadpoles, site, number of green tree frog tadpoles*site, number of cane toad tadpoles*site, number of green tree frog tadpoles*number of cane toad tadpoles, and number of green tree frog tadpoles*number of cane toad tadpoles*site.

Following MANOVA, we analysed data for each site separately using multifactor ANOVAs with survival, duration of larval period, body size at metamorphosis (length and mass) and growth rate as individual response variables. Factors assessed in ANOVAs for *R. marina* responses were number of green tree frog tadpoles and experimental block. Inclusion of the number of green tree frog tadpoles*block interaction resulted in the models failing to generate parameter estimates; therefore, we excluded the interaction term from these analyses. Factors included in ANOVAs for *L. caerulea* responses were number of green tree frog tadpoles, number of cane toad tadpoles, block, number of green tree frogs*block, number of cane toads*block, and number of green tree frogs*number of cane toads. Inclusion of the number of green tree frogs*number of cane toads*block interaction resulted in the models failing to generate parameter estimates, and therefore, we excluded this third-order interaction term. For all ANOVAs, we identified significant factors after sequentially removing non-significant higher to lower-order interactions, followed by non-significant block effects. With significant results, we performed *post hoc* Tukey's HSD tests to determine the location of variation. Analyses were performed using JMP 5.0.1 software (SAS Institute, Cary, NC).

RESULTS

Environmental conditions

The first week of the study experienced low temperatures due to heavy rains. Over the course of the study, Pond 1 experienced a higher mean temperature and greater diel variation (mean 28.41°C, range 18.5–35°C) than did Pond 2 (mean 26.8°C, range 24–30°C). Pond 1 dried out rapidly and was almost dry by the end of the experiment, whereas the depth of Pond 2 decreased slowly and was always greater than 60 cm. Throughout the experiment, water clarity was higher in Pond 1 (visibility to 1.5 m maximum depth) than in Pond 2 (visibility <10 cm).

Effects of green tree frogs, cane toad tadpoles and site

The overall MANOVA showed that the combined responses of survival, larval period, body length, body mass

and growth rate of cane toad larvae were significantly affected by experimental conditions (Wilks' lambda = 0.004, $F_{25,68} = 9.39$, $P < 0.0001$), with the number of green tree frog tadpoles ($F_{10,36} = 11.63$, $P < 0.0001$), site ($F_{5,18} = 49.73$, $P < 0.0001$) and the interaction between the number of green tree frog tadpoles and site ($F_{10,36} = 5.12$, $P < 0.0001$) all significantly affecting responses of cane toad larvae. The combined responses of green tree frog tadpoles were also affected by experimental conditions (overall MANOVA Wilks' lambda = 0.021, $F_{35,116} = 5.012$, $P < 0.0001$). The responses of *L. caerulea* were a function of the third-order interaction between number of *L. caerulea* tadpoles*number of *R. marina* tadpoles*site (MANOVA $F_{5,27} = 2.95$, $P = 0.03$). This significant highest-order interaction precluded interpretation of lower-order interactions and single factor effects in *L. caerulea* MANOVA analyses. As effects of site were significant for both species, we conducted further analyses for individual response variables at each pond separately.

Effects of competition on cane toads

In Pond 1, two of the five cane toad responses we measured were significantly affected by the number of green tree frog tadpoles in each treatment (duration of larval period, $F_{2,10} = 32.2$, $P < 0.0001$ and growth rate, $F_{2,10} = 46.27$, $P < 0.0001$; Figs 1c,3a). In Pond 2, four cane toad responses were significantly affected by the

number of green tree frog tadpoles (duration of larval period, $F_{2,12} = 19.57$, $P = 0.0002$; body length, $F_{2,8} = 6.53$, $P = 0.021$, block $P = 0.012$; mass, $F_{2,8} = 17.31$, $P = 0.001$, block $P = 0.001$; growth rate, $F_{2,12} = 51.13$, $P < 0.0001$; Figs 1c,2a,c,3a). At both sites, duration of the larval period was shorter and growth rate was faster in the absence of green tree frog tadpoles than in the presence of either 30 or 60 green tree frog tadpoles (Tukey $P < 0.05$; Figs 1c,3a). At Pond 2, exposure to green tree frog tadpoles also resulted in cane toads metamorphosing at reduced body length and mass; body length was reduced when toad tadpoles were exposed to 60 green tree frog tadpoles, and mass was reduced when toad tadpoles were exposed to either 30 or 60 green tree frog tadpoles (Tukey $P < 0.05$; Fig. 2a,c). Survival of cane toad tadpoles was not significantly affected by green tree frog tadpoles at either site (Fig. 1a).

Effects of competition on green tree frogs

In Pond 1, three of the five green tree frog responses were significantly affected by the number of con-specifics in each experimental treatment (survival, $F_{1,16} = 6.48$, $P = 0.021$; larval period, $F_{1,16} = 5.41$, $P = 0.034$; growth rate, $F_{1,16} = 8.85$, $P = 0.009$). Thus in Pond 1, a smaller number of conspecifics (30 rather than 60) resulted in increased green tree frog tadpole
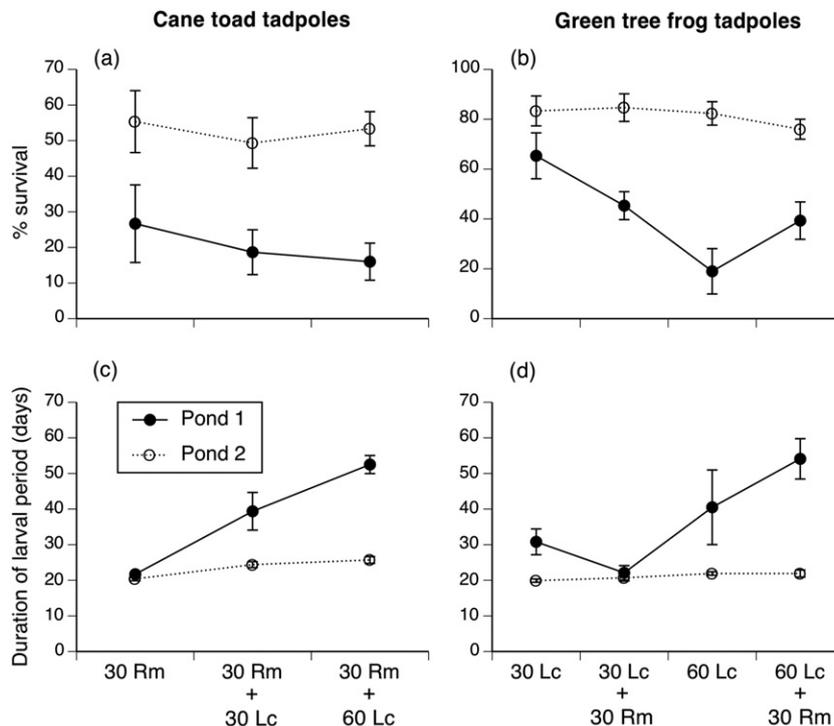

Fig. 1. Effects of experimental treatments on survival and duration of larval period of cane toad tadpoles (a,c) and green tree frog tadpoles (b,d) in two natural ponds. The figure shows mean values plus associated standard errors.
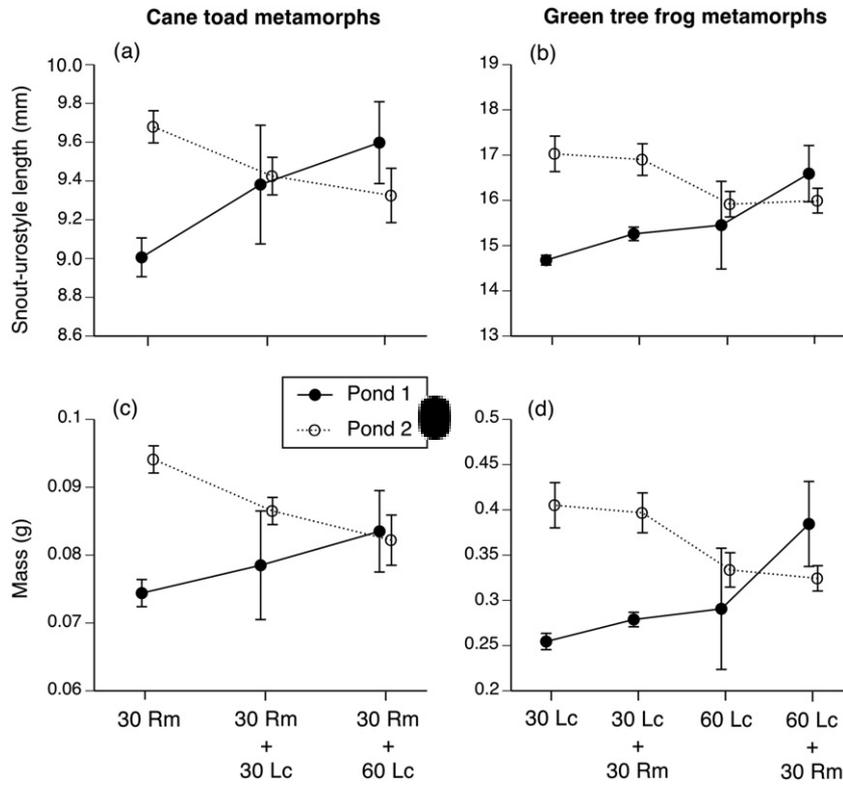
Fig. 2. Effects of experimental treatments on snout–urostyle length and mass of cane toad metamorphs (a,c) and green tree frog metamorphs (b,d) emerging from two natural ponds. The figure shows mean values plus associated standard errors.
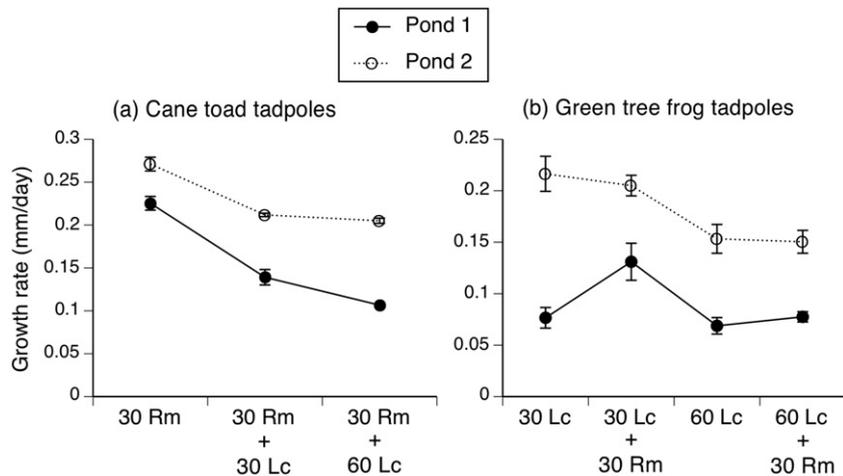

Fig. 3. Effects of experimental treatments on growth rates of cane toad tadpoles (a), and green tree frog tadpoles (b) in two natural ponds. The figure shows mean values plus associated standard errors.

survival and growth rate, and reduced larval period (Tukey $P < 0.05$; Figs 1b,d,3b). Effects on mass were marginally non-significant ($F_{1,16} = 4.43$, $P = 0.052$) suggesting a biological effect with a smaller number of conspecifics resulting in a decrease in mass at metamorphosis (Fig. 2d). The growth rate of green tree frogs also was affected by the number of cane toads in the treatments ($F_{1,16} = 8.65$, $P = 0.009$), but in a non-

intuitive direction. This effect was positive not negative: that is, frog tadpoles grew faster in the presence of cane toads (Tukey $P < 0.05$; Fig. 3b). The presence of cane toad larvae did not affect survival, larval period, size or mass at metamorphosis of green tree frogs in Pond 1 (Figs 1b,d,2b,d).

In Pond 2, four responses were affected by the number of conspecifics present in the enclosures (larval

period, $F_{1,13} = 11.21$, $P = 0.005$, block $P = 0.027$; body length, $F_{1,13} = 111.28$, $P < 0.0001$, block $P < 0.0001$; mass, $F_{1,13} = 79.92$, $P < 0.0001$, block $P < 0.0001$; growth rate, $F_{1,13} = 115.88$, $P < 0.0001$, block $P < 0.0001$). Higher density of conspecifics resulted in reduced size of the metamorphs (body length and mass), increased larval period and reduced growth rate. The presence of cane toad tadpoles did not affect survival, larval period, metamorph size or growth rate of green tree frog tadpoles in Pond 2 (Figs 1b,d,2b,d,3b).

DISCUSSION

Do larval cane toads and green tree frogs compete in nature?

Tadpoles of cane toads and green tree frogs often co-occur in natural waterbodies. Our field experiments did not reveal any effects of tadpoles of green tree frogs (*Litoria caerulea*) on the survival of cane toad (*Rhinella marina*) tadpoles, or vice versa. However, the duration of larval period and the growth rate of cane toad larvae were negatively affected by the presence of green tree frog tadpoles in both ponds; body length and mass were also negatively affected in Pond 2. Frog tadpoles either were not affected in these respects by toad tadpoles or (in the case of growth rate of green tree frog tadpoles in Pond 1) were affected in a positive manner.

Under field conditions, Alford *et al.* (1995) reported a mean time for development of cane toads from the end of the hatchling stage to metamorphosis of about 38 days. In our study, toad tadpoles were added to experimental cages 1 to 2 days after the end of the hatchling stage. Despite these 1 or 2 days not being included in our calculation of larval period (see *Experimental protocol*), it is nonetheless apparent that, in the absence of green tree frog tadpoles, development was faster than the time reported by Alford *et al.* (perhaps reflecting the high ambient temperatures of our study area): the average time to metamorphosis of toads was 22 days in the field in Pond 1 and 21 days in Pond 2. In treatments containing green tree frog tadpoles, this time increased, especially in Pond 1 (39 and 52 days with 30 and 60 green tree frogs respectively, and 25 and 26 days in Pond 2 with the same densities). Thus, the growth rate of cane toads was slower in the presence of either 30 or 60 green tree frog tadpoles, and this effect was greater in Pond 1 than in Pond 2. This competition-induced decrease in growth rate, and consequent increase in larval period, has important biological implications. Many of the temporary ponds used as spawning sites by cane toads evaporate entirely by the end of the wet season (as, indeed, was the case for Pond 1 in the year of our study). The duration of inundation of these ponds ranges from 10 days to 3 months in our study area (M. R. Crossland & E. Cabrera-Guzmán, pers. obs.,

2010), and thus, prolonging the larval phase may result in very high mortality rates if metamorphosis cannot be completed before the pond dries out.

A longer larval period also increases the time that tadpoles are subject to other mortality factors in the aquatic environment (DeBenedictis 1974). In the case of cane toad larvae, for example, predation by aquatic invertebrates can be intense (Cabrera-Guzmán *et al.* 2012). Additionally, slower-growing anuran larvae may be less likely to survive, because smaller animals can be deprived of food by competition from larger ones, even if smaller animals have lower individual food requirements (Schoener 1983; Persson 1985). Growth rate can thus be directly related to food intake for anuran larvae (Bardsley & Beebee 2000). Even if slow-growing cane toad tadpoles do complete larval development in the presence of green tree frog tadpoles, our results show that interspecific competition can result in significantly reduced cane toad size at metamorphosis (e.g. Pond 2). This reduced size may have significant fitness consequences because, in amphibians, body size in early terrestrial life history stages is positively correlated with subsequent survival (Berven & Gill 1983; Smith 1987) and reproductive output (e.g. Clarke 1974; Howard 1978a,b; Wilbur *et al.* 1978). In the case of cane toads specifically, smaller metamorphs have a greater risk of mortality due to desiccation (Child *et al.* 2008), predation (Ward-Fear *et al.* 2010b) and parasites (Kelehear 2007). The negative effects of tree frog tadpoles presence on cane toad larvae development and size at metamorphosis demonstrated in this study thus suggest that competition with native frogs may reduce recruitment of toad populations in nature.

The mechanism of competition between green tree frog tadpoles and cane toad tadpoles remains unclear, and is the subject of our continuing studies. The difference between ponds in the magnitude of the competitive effects on cane toad tadpoles, and the greater effect of high conspecific density of green tree frog tadpoles in Pond 1 with lower productivity, suggest direct exploitative competition for food. However, anuran larvae also can affect each other's viability by behavioural interactions, suppression by chemical–biological substances or by interactions with the unicellular alga *Prototheca richardsi* (Beebee 1991; Griffiths *et al.* 1991, 1993). The competitive superiority of green tree frog tadpoles may at least partly be a function of their large size relative to cane toad tadpoles, because larger anuran tadpoles often outcompete smaller tadpoles (e.g. Steinwascher 1978; Morin & Johnson 1988; Werner & Anholt 1996; Kupferberg 1997a).

How do environmental conditions in breeding ponds affect tadpoles?

Variation in rainfall across years determines the numbers of ephemeral breeding ponds, and their

duration in the Australian wet–dry tropics (Shine & Brown 2008). In turn, pond persistence, and characteristics such as temperature and food availability, can affect the survival, growth and development of amphibian larvae (e.g. Petranka 1984; Holomuzki 1986; Ultsch *et al.* 1999; Álvarez & Nicieza 2002). Consistent with that sensitivity, we detected significant differences between our two study ponds in tadpole survival, larval period, size, mass and growth rate, in both of our study species. The low rate of survival of larval cane toads and green tree frogs at Pond 1, in all treatments, likely was due to the low productivity (and thus, low food availability) of this clear-water sandy system. Reduced viability of cane toad tadpoles at this pond presumably was a direct result of reduced feeding rates, but the effects on tree frog tadpoles may have been either direct (i.e. the same as for toads) or indirect. Tree frog tadpoles readily eat dead toad tadpoles but do not pursue and kill live toad tadpoles, and thus, starvation-induced mortality of toads would induce carnivory by tree frog tadpoles, which would then be killed by lethal toxins in the dead toads (Crossland & Azevedo-Ramos 1999; Hayes *et al.* 2009).

Environmentally induced plasticity in the duration of larval period, body length, mass, shape and growth rate is common in amphibian larvae, as a function of abiotic variables in the waterbodies (e.g. temperature, canopy cover, waterbody duration) and food resources (e.g. nutritional variation among algal taxa, abundance of algae, leaf litter type, composition of aquatic vegetation: Tejedo & Reques 1994; Kupferberg 1997b; Álvarez & Nicieza 2002; Williams *et al.* 2008; Van Buskirk 2009; Walsh 2010). For larval anurans, higher water temperatures generally result in faster development and smaller size at metamorphosis (Blaustein *et al.* 1999; Álvarez & Nicieza 2002). However, tadpoles in our study showed a different pattern: larvae in the warmer pond took longer to complete development, with metamorphosis delayed for a month in Pond 1. Counter-intuitively, however, longer larval periods were associated with the production of larger not smaller metamorphs. That result mirrors reports that reduced rates of growth and prolongation of larval period in tadpoles raised at low temperatures (as recorded in this pond) can be accompanied by relatively large size at metamorphosis (Atkinson 1994; Álvarez & Nicieza 2002). In Pond 1, any such direct effect may have been enhanced by the decrease in the number of competing animals in enclosures across time, the frequent relocation of cages within the ponds (potentially providing access to unexploited food resources) and the higher concentration of nutrients as the pool shrank in size throughout the experiment.

Overall, the primary outcome from our study is to show that the strong competitive suppression of cane toad tadpoles by tree frog tadpoles (as observed in our previous laboratory study: Cabrera-Guzmán *et al.* 2011) is not an artefact of unrealistic laboratory conditions. At realistic densities, the larvae of native frogs and cane toads compete in natural ponds. The tadpoles of cane toads were more affected by the presence of native tadpoles (or at least, of *L. caerulea* tadpoles) than vice versa, supporting previous suggestions that cane toad tadpoles are relatively poor competitors (Alford 1999; Smith 2005). Although the effects of *L. caerulea* on the survival of cane toad larvae were not as strong under natural conditions as they were in the laboratory (see Cabrera-Guzmán *et al.* 2011), the effects on duration of larval period, growth rate and size at metamorphosis in natural ponds may well influence cane toad recruitment. Our data also suggest that the effects of tadpole competition depend upon the characteristics of the ponds. Our results support the hypothesis that invasive cane toads encounter biotic resistance from native frogs in Australia (see also Alford 1999; Crossland *et al.* 2009), and demonstrate that this resistance can occur under natural conditions. Competition for prey items between toads and frogs may be minimal in the terrestrial phase of the life history (Greenlees *et al.* 2007), but competition between their larvae may be more significant.

Clearly, much remains to be learnt about these interactions and their spatial and temporal variability. For example, in addition to size advantages, tadpoles of earlier breeding anurans also may gain a competitive advantage over later breeding species via nutrient depletion through consumption by early arriving species, or alteration of pond trophic structure (Alford & Wilbur 1985; Wilbur & Alford 1985; Alford 1989a). Therefore, in ponds where breeding of green tree frogs precedes that of cane toads, the negative effects of the native species on toads may be even greater than documented in this study (where tadpoles of both species were added to experimental cages simultaneously, rather than sequentially). However, such priority effects are not always predictable (Alford & Wilbur 1985; Alford 1989b). Other factors potentially influencing competitive outcomes among ponds include the presence of predators (Morin 1981; Wilbur 1987), the distribution of food resources (Kiesecker *et al.* 2001) and variation in water chemistry (Warner *et al.* 1993). The current experiment relied upon mesh enclosures. Ideally our experiment should be replicated at a whole-pond level, that is, to compare toad recruitment from ponds containing tree frog larvae with those that contain only toads. Such trials are logistically difficult, but may not be impossible. At our study site, cane toads spawn most frequently at the end of the wet season, as pond levels begin to fall, whereas most native frogs spawn at the height of the wet season rains a month or two previously (M. R. Crossland, pers. obs., 2010). The willingness of toads to spawn in ponds already containing the larvae of native frogs such as tree frogs may be an 'evolutionary trap' (*sensu* Schlaepfer *et al.* 2002). That is, the lack of a long coevolutionary history between these two types of

anurans means that there has been insufficient time for effective selection on toads to detect and avoid ponds already containing frog tadpoles. In the same way, cane toads do not recognize and avoid large carnivorous ants that feed heavily upon metamorph toads in Australia (but not within the toads' native range; Ward-Fear *et al.* 2009), and adult toads are readily attracted to a wriggling caudal lure of a predatory Australian snake (Hagman *et al.* 2009).

Our study is encouraging for the idea that healthy populations of native frogs likely confer some level of biotic resistance against cane toads. That is, toads may be less likely to thrive in areas that already contain dense populations of larval anurans in suitable waterbodies. Spreading this message to the general public could encourage rehabilitation of degraded peri-urban wetlands, and thus achieve many conservation benefits other than simply reducing cane toad recruitment.

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