Sexual selection in cane toads *Rhinella marina*: A male’s body size affects his success and his tactics

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Abstract Male body size can play an important role in the mating systems of anuran amphibians. We conducted laboratory-based trials with cane toads *Rhinella* (*Bufo*) *marina* from an invasive population in the wet-dry tropics of northern Australia, to clarify the effects of a male's body size on his reproductive success and behavior (mate choice). Males were stimulated with a synthetic hormone to induce reproductive readiness. Larger body size enhanced a male toad's ability to displace a smaller rival from amplexus, apparently because of physical strength: more force was required to dislodge a larger than a smaller amplexant male. A male's body size also affected his mate-choice criteria. Males of all body sizes were as likely to attempt amplexus with another male as with a female of the same size, and preferred larger rather than smaller sexual targets. However, this size preference was stronger in larger males and hence, amplexus was size-assortative. This pattern broke down when males were given access to already-amplectant male-female pairs: males of all body sizes readily attempted amplexus with the pair, with no size discrimination. An amplexant pair provides a larger visual stimulus, and prolonged amplexus provides a strong cue for sex identification (one of the individuals involved is almost certainly a female). Thus, a male cane toad’s body size affects both his ability to defeat rivals in physical struggles over females, and the criteria he uses when selecting potential mates, but the impacts of that selectivity depend upon the context in which mating occurs [Current Zoology 59 (6): 747–753, 2013].

Keywords Mating systems, Size-assortative mating, Large-male advantage, Anurans, Invasive species

In many species, an animal’s body size influences its reproductive behavior and success. For example, larger females may produce larger or more numerous offspring than small females (Blueweiss et al., 1978), and larger males may dominate their rivals and thus obtain more mates (e.g., harlequin beetles: Zeh et al., 1992; wrens: Haggerty, 2006; brown bears: Zedrosser et al., 2007). Such advantages of larger body size in males may favor size-dependent shifts in reproductive tactics. For example, smaller males may rely upon 'sneaking' rather than territorial defence (e.g., Gross, 1996; Thompson et al., 1993) or attempt to intercept females that are attracted to their more dominant rivals (Forester and Lykens, 1986; Leary et al., 2005). More generally, larger body size may enable a male to achieve greater success than his smaller rivals (either by mating more often, or by mating with larger and thus more fecund females). Similarly, a male’s body size may influence his tactics (e.g., criteria for mate choice) in ways that exploit those physical advantages.

Bufonid anurans have provided popular study species for analyses of sexual tactics, reflecting the cosmopolitan distribution and abundance of toads. However, tropical toads have attracted less research in this respect than have temperate-zone species (e.g., Aronson, 1944; Davies and Halliday, 1977; Sullivan, 1984; Höglund and Robertson, 1988). Mating systems (and thus, the role of male body size for reproductive tactics and success) vary considerably among toad species. In many bufonids, female choice is more important than any physical interactions between rivals in determining male mating success (e.g., Heatwole, 1995; Wells, 2007; Sullivan and Kwiatowski, 2007) whereas in other toad taxa, males fight each other vigorously for access to reproductive females (e.g., Davies and Halliday, 1978; Vargas-Salinas, 2007). We investigated the role that body size plays in the mating system of cane toads *Rhinella marina* from an invasive population in the wet-dry tropics of Australia. Specifically, we explored whether or not a male toad’s body size influences his success in male-male rivalry (e.g., in obtaining amplexus, and in maintaining that position despite attempts to dislodge him) and the cues (including size and sex of a potential partner) that he uses to select potential mates.
1 Materials and Methods

1.1 Animal collection and morphological measurement

We collected adult cane toads from the Adelaide River floodplain in the Northern Territory of Australia (12°38’S, 131°19’E) in January–February 2006. We individually marked all animals with a unique number on flagging tape, tied around the ‘waist’. We measured mass (to the nearest 0.5 g) and snout-urostyle length (SUL, to the nearest mm) in all animals. To induce reproductive behavior, we gave each ‘response male’ (see below) a subcutaneous 0.5 mL injection of leuprorelin acetate (Lucrin®, Abbott Australasia, Kurnell, Australia) as 0.25 mg mL⁻¹ in amphibian Ringer’s solution, at least 3 hours before the trials began. Leuprorelin acetate mimics the action of gonadotropin releasing hormone (GnRH), which stimulates gonadal production of sex steroids and thus, stimulates reproductive behavior (Propper and Dixon, 1997). Although our results thus may not mimic male behaviors in the wild, the procedure should not induce any artifacts to confound interpretation of the present study (e.g., it will not generate spurious correlations between a male’s body size and his strength or mate-selection tactics), as all ‘response’ males were subject to identical treatments of leuprorelin acetate. Following injection, we housed males communally with excess females in large plastic tubs (1.09 × 1.09 × 0.63 m, filled with 10 L water, plus hay and rocks, in an outdoor area) to stimulate amplexus behavior (Wells, 1977). Our trials used males that we observed in amplexus in the communal tub, to ensure we only used reproductive males. Performance in ectotherms frequently correlates with temperature (Huey and Stevenson, 1979), so we analyzed ambient thermal data from a nearby site (Bureau of Meteorology, http://www.bom.gov.au); temperature was not correlated with the dependent variables in any data set, so we excluded it in all analyses. We conducted all trials at night, under red lighting, to minimize visual disturbance (Peterson et al., 1992). Below, we refer to males from which we scored a response as ‘response’ males, whereas the males and females that were placed with them (to elicit reproductive behavior) will be referred to as ‘stimulus’ males and females.

1.2 Does a male’s body size influence his ability to obtain amplexus?

In natural breeding aggregations of some (but not all) bufonid species, adult males greatly outnumber females (Wells, 1977, 2007). This situation is common in cane toads (Lever, 2001; Vargas-Salinas, 2007). In such systems, a single female toad may be seized by more than one male, and males often attempt amplexus with females that are already being amplexed by rival males (e.g., Darwin, 1871; Davies and Halliday, 1979; Vargas-Salinas 2007 shows a photograph of three males trying to amplex a single female in a field aggregation of cane toads). To mimic this situation in the laboratory, we conducted trials with three response males and a single stimulus female. The males represented three size classes, with at least 30 g mass and/or at least 5 mm SUL difference between each class (total size range of males, 90 to 270 g, where class 1, mean mass = 105.7 ± 1.8 g and mean SUL = 103.0 ± 0.9 mm; class 2, mean mass = 143.9 ± 2.3 g and mean SUL = 115.2 ± 0.9 mm; and, class 3, mean mass = 196.6 ± 6.0 g and mean SUL = 123.7 ± 1.1 mm). We introduced three amplexant males (plus their female partners) into a breeding arena (plastic tub, dimensions as above). We then conducted 17 trials, each consisting of introducing a single stimulus female into the arena, with the female initially held within a transparent plastic container in the middle of the arena to ensure that she was equidistant to all males at the beginning of a trial. We separated each male from amplexus (removing the amplexant females), and then immediately released the stimulus female from beneath the transparent container. We observed resultant behaviors for 5 minutes, noting which male amplexed the female first, if there were any challenges to the amplexant male and if so, which size class of male was victorious. We defined a ‘challenge’ as any male-male interaction whereby an unpaired male attempted to amplex a pair. The animals were then left undisturbed as we repeated the 5-minute observation session after 30 min, 60 min, 120 min and at 0600 h the following morning (approx. 9 hours after commencement of the experiment). Each animal was used in only a single trial.

1.3 Does a male’s body size influence his ability to maintain amplexus?

Prior to the trials, we anaesthetized one small female (mass 105 g, SUL 105 mm) and one large female (mass 194 g, SUL 132 mm) by immersing them in a bath of methanotricaine sulphate. Each female was then firmly attached to a plastic substrate using cable-ties around her body near the fore- and hindlimbs. We also attached a cable-tie around the midregion (‘waist’) of each amplexant male, to allow attachment of a Pesola® spring scale. For each trial, we selected an amplexant male from the communal tub, separated the male from his partner, and positioned him on top of the prostrate female. In every case, the male immediately amplexed the immobile female. We then hooked the spring scale to
the male’s cable tie and pulled directly upwards, so that we could record the force required for dislodgment (male tenacity) as the maximal reading on the scale (i.e., when the male was dislodged). To investigate influences of male-female size relationships on male tenacity (e.g., whether small males hold onto small females more strongly than on large females, or vice versa), we tested the tenacity of each male on both small and large females (with 1 hour break for each male in between trials). To account for any effects of trial order, we reversed the order on successive trial nights.

1.4 Does a male’s body size influence his criteria for mate choice?

We conducted two sets of trials to clarify the basis for mate choice by males, in a situation (as may occur in the field, when a female toad approaches a male near the edge of a waterbody) where a male has access to visual cues relating to a conspecific’s sex, body size, and whether or not it is already in amplexus, and its relative body size. One set of trials exposed a male to a single stimulus animal, whereas the other set involved multiple potential partners.

(a) Mate choice in a single stimulus arena. – During a parallel study where we conducted acoustic playback experiments to clarify the proximate determinants of amplexus duration (see Bowcock et al., 2008, for a full description of this study), we paired a response male with a single stimulus toad (male or female) in a plastic enclosure measuring 0.60 × 0.39 × 0.35 m. The data from these trials were used to assess whether the body size of the stimulus toad influenced the probability that amplexus would be initiated.

(b) Mate choice in an arena with multiple stimulus animals. – Methods for the mate choice trials were modified from Aronson (1944), and conducted in a large plastic tub as described above. Stimulus animals were four solitary males, four solitary females, and four amplexant (male plus female) pairs. For each trial (56 in total), we selected an amplexant pair from the communal breeding arena, separated the male and immediately introduced him into the choice arena. We introduced the response male at the opposite end to stimulus animals (which were localized at one end of the tub), so that the response male was a similar distance from all of the stimulus animals at the beginning of each trial. We scored which stimulus (i.e., solitary male, solitary female or pair) was first amplexed (clasped) by the response male (stimulus males never approached the response males or the stimulus females, so did not need to be scored). Trials were terminated when the response male amplexed another toad, or after 5 minutes (whichever came first).

1.5 Data analysis

In the male-male interaction trials, we used a $\chi^2$ goodness-of-fit test against a null expectation of equal numbers of victories to smaller and larger males (using VassarStats: Lowry, 2007).

For analysis of male tenacity we performed repeated-measures ANOVA in Statview v5.0 (SAS Institute, Cary, North Carolina) using the force required for dislodgement as the dependent variable, with male size class (‘small’ ≤100 mm, ‘medium’ 101–106 mm, and ‘large’ ≥107 mm) as the factor and female body size (small vs. large female) as the repeated measure. Because male mass alone (and not order of trial by female size, nor any interaction) was the most important correlate of variation in tenacity (all other $P > 0.05$), we regressed mean tenacity (averaged over the two female sizes) against male mass using linear techniques in JMP v5.0.1a (SAS Institute, Cary, North Carolina).

In the multiple stimuli trials we compared numbers of amplexus attempts using $\chi^2$ goodness-of-fit tests in VassarStats (Lowry, 2007). We compared amplexus and non-amplexus responses for the single and multiple choice experiments using logistic regression in JMP v5.0.1a. We used response and stimulus animal mass, and stimulus sex (‘male’ and ‘female’ in the single stimulus experiments, but ‘male’, ‘female’ and ‘pair’ in the multiple choice experiments) as independent variables, after deleting any non-significant interaction terms from the models. For the occasions where male toads attempted amplexus, we regressed response against stimulus body size in the single stimulus (by mass) and the multiple stimuli (by SUL) trials, using linear techniques in JMP. In the multiple stimuli experiments, we performed two-factor ANOVA in JMP v5.0.1a, with stimulus SUL as the dependent variable, with response male SUL and stimulus identity as independent variables.

2 Results

2.1 Does a male’s body size influence his ability to obtain amplexus?

Larger males were more successful in displacing smaller males than vice versa (winning 10 of 12 encounters, $\chi^2 = 5.33$, $P = 0.03$).

2.2 Does a male’s body size influence his ability to maintain amplexus?

Large males were more tenacious, requiring almost twice as much force to be dislodged as did smaller
males (Fig. 1; body mass vs. mean force required for dislodgement, \( n = 34, r^2 = 0.34, P = 0.0002 \)). ANOVA with male size class as the factor and female body mass as the repeated measure revealed a strong effect of male body size on tenacity \( (F_{2,32} = 9.64, P < 0.001) \), but no significant effect of female body size \( (F_{1,32} = 1.34, P = 0.26) \) and no significant interaction between male and female sizes \( (F_{2,32} = 0.79, P = 0.46) \).

**Fig. 1** Body size influence on tenacity in cane toads *Rhinella marina*

Larger males were more tenacious than smaller males i.e., the force required to dislodge an amplectant male from a female was greater for larger males than for smaller males.

2.3 Does a male’s body size influence his criteria for mate choice?

When presented with a single stimulus animal, whether or not a male toad amplexed that animal was affected by the mass of the stimulus toad, but not its sex (Table 1). Males were more likely to amplex larger stimuli (regression of probability of amplexus vs. stimulus mass: \( n = 156, r^2 = 0.78, P < 0.0001 \)). Larger males were just as likely to attempt amplexus as were small males (Table 1), but male body size influenced the ‘target’ selected for amplexus: larger males tended to amplex larger targets. The correlation was significant for body mass, albeit with wide scatter around the relationship (Fig. 2A; \( n = 122, r^2 = 0.04, P = 0.02 \)).

When there were multiple stimuli available, most approaches by male toads resulted in attempts at amplexus (5 approaches without amplexus attempts vs. 48 amplexus attempts). Our analyses suggest that male toads did not utilize cues reflecting the sex or status (paired vs. solitary) of potential targets when selecting a partner for amplexus. Although males attempted amplexus more frequently with pairs than with solitary males and females, this bias fell short of statistical significance (23 vs. 12 vs. 16 cases, respectively; \( \chi^2 = 3.22, P = 0.20 \)). A male toad’s response was affected by the mass of the stimulus toad relative to his own mass (significant interaction between response and stimulus mass: Table 1). As in the single-stimulus trials, larger males that attempted amplexus with a solitary sexual ‘target’ (of either sex) tended to select larger-bodied ‘targets’, resulting in size-assortative amplexus with respect to snout-urostyle length (Fig. 2B; \( n = 27, r^2 = 0.17, P = 0.034 \)). The ‘targets’ that were clasped while already amplexent were smaller, on average, than the ‘targets’ selected when solitary (means of 109 vs. 117 mm SUL, two-factor ANOVA; \( F_{1,45} = 4.55, P = 0.001 \)).

This readiness to amplex even small toads if they were already part of an amplectant pair was evident for large as well as small males. As a result, the mean sizes of ‘target’ toads were similar to those attempted with solitary amplexent were, on average, than were the ‘targets’ selected when solitary (means of 109 vs. 117 mm SUL, two-factor ANOVA; \( F_{1,45} = 5.61, P < 0.025 \)).

**3 Discussion**

Despite an extensive published literature on cane toads (e.g., see Lever’s 2001 monographic review of this taxon), its sexual behavior remains poorly known (but

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In the multiple stimuli arena, ‘Stimulus sex’ categories are single male, single female and pair. ‘Stimulus mass’ refers to the mass of the toad that is amplexed by the male, while ‘Response mass’ is the mass of the amplectant male. ‘Stimulus*Response mass’ is the interaction term, which was only included in the analysis if it was significant in stepwise analysis.
see Vargas-Salinas, 2007). Ideally, field observations are needed; but for logistical reasons, we restricted our work to hormonally-primed male toads in outdoor enclosures. Our trials show that a male toad's body size affects the tenacity with which it can cling to a female during amplexus, regardless of female body size; and perhaps for this reason, larger males are able to dislodge smaller males in physical battles for the amplexant position on the female. A male's size also affects his criteria for mate choice (larger males tend to select and amplex larger females), but in a flexible way (that size-selectivity disappears if the 'target' animal is already in amplexus when it is encountered, perhaps because of male inability to accurately judge female size in that circumstance).

Most of these patterns are straightforward, and it is easy to suggest potential causal mechanisms. Although male bufonids of many species rarely if ever engage in physical combat over reproductive females (e.g., Heatwole, 1995; Wells, 2007), a large-male advantage in amplexus takeovers has been reported in other explo- sively-breeding bufonids (Davies and Halliday, 1979; Lamb, 1984; Telford and van Sickle, 1989). That size advantage presumably results from increased physical strength. Larger males can hang on more effectively regardless of female body size, and thus are able to usurp the amplexant position of smaller males. Alternatively, our experimental manipulation (lucrin injection to stimulate reproductive behaviour) may have had differential effects on motivation levels of males of different body sizes. Although we doubt this possibility (all males received equal doses of lucrin), it would be useful to repeat our studies with toads in field situations where breeding is occurring.

In terms of mate choice, all males may benefit from amplexing larger rather than smaller females because in cane toads (as in many anuran species), larger females tend to produce more eggs (e.g., Wilbur et al., 1978; Tejedo, 1992; Lampo and Medialdea, 1996; Castellano et al., 2004; Camargo et al., 2005), thereby increasing the fitness benefits of mating for the male. Also, because female cane toads grow larger than males (Lee, 2001; Lever, 2001), a larger animal is more likely to be a female. However, male preference for larger females means that such animals are likely to attract multiple suitors, especially if the operational sex ratio is highly male-biased (as is often the case in nature: Lever, 2001). Hence, only males large enough to withstand physical attack by rivals are likely to remain in position for long enough to obtain the reproductive outcome. This process would increase the fitness benefits of size-assortative courtship, as observed in both sets of our laboratory trials. Additionally, lower fertilization success if a small male mates with a large female, due to reduced proximity of their vents (e.g., Lengagne et al., 2007) may favour a preference by smaller males for smaller females. The fitness consequences of male mate choice may be even greater if male reproductive potential is limited, thus restricting the number of potential matings per male (Hettyey et al., 2009). The fitness benefits of size-assortative courtship for females (if any) remain unclear for this system.

The inability of males to discriminate conspecific sex by visual cues is consistent with findings for other bufonids (Marco et al., 1998; Marco and Lizana, 2002). While mistakes can be (and are) made in a size-based

**Fig. 2** Relative body sizes of amplexant male ('response male') and clasped individual ('stimulus anuran') of cane toad *Rhinella marina* pairs during mate choice trials
In (A), relative body sizes (mass) are from trials in which males formed pairs when presented with a single stimulus. In (B), relative body sizes (snout-urostyle lengths) are from trials in which males formed pairs with a single stimulus toad from a selection of stimuli (four each of single males, single females and amplexant pairs). In both sets of trials, toads paired in a size-assortative manner.
mate selection strategy, the cane toad mating system may have provided little selective pressure for pre-amplexus sex recognition in males. At high densities, where breeding is explosive, speed may be favored over efficiency in mate acquisition, as the benefits of being first to clasp a female outweigh costs of erroneously clasping males (Wells, 1977). By clasping any toad that is larger than themselves, males are more likely to locate females (see above). If a large male is erroneously clasped, he can give an acoustic signal that initiates release from amplexus (the release call: Aronson, 1944; Bowcock et al., 2008).

Why do males of all size classes direct amplexus attempts towards small as well as large 'targets' if those 'targets' are already in amplexus when encountered? The most likely explanation is confusion: the body size of a toad is difficult to evaluate if the female is tightly clasped by another toad, and the visual stimulus presented by two tightly linked toads may resemble that provided by a single large animal. Interestingly, that apparent difficulty of discriminating female body size in amplexed animals has more effects on 'target' size for large male toads than for smaller males (which typically attempt to amplex small as well as large 'targets' anyway; Fig. 2B). Regardless of any difficulty of evaluating body sizes, an already-amplectant pair presumably contains a female (most male-male amplexus attempts terminate quickly: Bowcock et al., 2008) and thus, amplexus provides a strong cue for sex identification. Such reliance upon courtship activities of other males to locate females may be widespread in animal populations with highly male-skewed operational sex ratios in the breeding area (e.g., see Shine and Mason, 2001 for similar results on garter snakes Thamnophis sirtalis).

Size-assortative amplexus was evident in both sets of our laboratory trials (i.e., with both single and multiple stimuli), and it would be interesting to know whether the same pattern exists in natural populations. Extensive research on this topic in anurans (e.g., Gatz, 1981; Kruse, 1981; Gutierrez and Ludecke, 2002; Friedl and Klump, 2005), shows that size-assortative mating is common but not ubiquitous, and can vary even among conspecific populations, or within the same population through time (Wilbur et al., 1978; Olson et al., 1986). The degree to which larger male body size enhances breeding success is similarly variable (Wilbur et al., 1978; Wagner and Sullivan, 1995), and is complicated by the fact that the locomotor costs to the female of maintaining amplexus increase with male size (Bowcock et al., 2009). Some authors have attributed size-assortative mating to intrasexual selection (the largest males are the strongest, and most able to obtain the largest and most fecund females: Davies and Halliday, 1978; Howard, 1981) whereas others have advocated a role for intersexual selection. Such female choice on male body size might relate to size-matching (reflecting the advantages of close cloacal apposition for effective fertilization of the eggs: Licht, 1976; Davies and Halliday, 1977) or simply that large males represent individuals of high fitness, and larger females are somehow able to selectively mate with such animals (Wilbur et al., 1978; Davies and Halliday, 1979).

Our results concerning mate choice and the role of male body size suggest profitable lines of further enquiry into sexual selection in cane toads. Anuran mating systems can vary considerably through time as well as space and phylogeny (see above), though few populations have been studied over more than a few seasons (but see Olson et al., 1986). Observations of free-ranging cane toads in South America (Vargas-Salinas, 2007) suggested random mating, contrasting with the mating patterns observed in our own study. This contradiction highlights the need for our laboratory studies to be extended into observations of natural populations in the field, in a range of populations and over a number of seasons.

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References
Castellano S, Cucco M, Giacoma C, 2004. Reproductive invest-


