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Why be diurnal? Shifts in activity time enable young cane toads to evade cannibalistic conspecifics

Lígia Pizzato, Travis Child, and Richard Shine
School of Biological Sciences A08, University of Sydney, New South Wales 2006, Australia

Why are some animals active by day and others by night? The selective forces that favor diurnal versus nocturnal activity may be evaluated by comparing age classes within a species that exhibits intraspecific (ontogenetic) variation in activity times. In many species of toads, adults are nocturnally active but postmetamorphic animals are primarily diurnal. The small body sizes of these animals render them vulnerable to desiccation and overheating—so why are they active by day? To answer this question, we studied an invasive population of cane toads (*Bufo marinus*) in tropical Australia. In the field, these small toads often encounter cannibalistic conspecifics because desiccation risk concentrates toads around the moist margins of the natal pond. We manipulated factors that differ between day and night (time of day, illumination, presence of cannibalistic conspecifics, scent, or visual cues from cannibalistic conspecifics) to identify the proximate cues and fitness advantages associated with diurnal versus nocturnal activity. Activity levels, response to disturbance, and feeding rates of metamorph toads were enhanced by light but suppressed by the presence of a larger conspecific. Metamorphs used both visual and scent cues to detect larger toads. An endogenous diel rhythm in activity was present also but weaker in metamorph toads than in larger (cannibal sized) individuals. The risk of cannibalism was high only at night and only in dark conditions. Thus, the diurnal activity of metamorph toads enables these vulnerable animals to avoid conspecific predators. *Key words:* activity patterns, anuran, cannibalism, diel rhythm, predation. *[Behav Ecol 19:990–997 (2008)]*

The times of day that an animal is active constitute an important dimension of its ecological niche (see Schoener 1974; Albrecht and Gotelli 2001; Kocárek 2001; Kronfeld-Schor and Dayan 2003). Different patterns of diel activity expose an animal not only to different abiotic conditions (e.g., temperature, humidity) but also determine the effectiveness of alternative sensory modalities (e.g., illumination levels influence the reliability of color vision: Dyer and Chittka 2004; Melamud et al. 2004), the kinds of prey that are available, and the types of predators to which the animal may be exposed (Halle 1993; Angeli et al. 1995; Metcalfe et al. 1999). Thus, sympatric diurnal and nocturnal organisms may experience profoundly different environments (Metcalfe et al. 1999; Oishi et al. 2004; Dar et al. 2006).

Many factors may influence activity times, so how can we best identify proximate mechanisms and ultimate (adaptive) advantages of specific diel activity schedules? The best opportunities may come from taxa that display lability in times of activity. For example, the diel timing of activity may shift seasonally (e.g., snakes—Shine 1987; Schwaner 1989; fishes—Heggenes et al. 1993; Sanchez-Vazquez et al. 1998; salamanders—Dolmen 1983a, 1983b; frogs—Oishi et al. 2004), geographically (e.g., reptiles—Cogger 1983; flies—Dahlgaard et al. 2001; salamanders—Dolmen 1983a, 1983b; lions—van Orsdol 1984), or in response to anthropogenic disturbance (e.g., coyotes—Kitchen et al. 2000). Even better, in some taxa, the diel rhythms of activity differ between subgroups within a single population (by sex, size, age, hierarchical status, or state: Dolmen 1983a, 1983b; Magnan and Fitzgerald 1983; van Burskirk 1992; Metcalfe et al. 1998; Alanärä et al. 2001; Marcelli et al. 2003; Hansen and Closs 2005; Rudolf 2006; for reviews, see Kronfeld-Schor and Dayan 2003; Neuhaus and Ruckstuhl 2005). Such intraspecific diversity facilitates direct comparisons between individuals that are similar in most other respects and live in the same place at the same time.

Two kinds of information on such taxa can be used to clarify intraspecific divergence in times of activity. First, we can explore the advantages and disadvantages of activity at different times to identify selective forces that may have shaped that divergence in activity schedules. Second, we can identify proximate cues that drive activity patterns; such cues may not only provide direct information on underlying selective forces but also allow us to predict how variation in ambient conditions (proximate cues) will translate into variation in schedules of activity.

Although they are largely nocturnal as adults, many anuran amphibians (frogs and toads) display occasional diurnal activity. In some species, diurnal basking serves a thermoregulatory function (Brattstrom 1979; Hutchinson and Dupre 1992; Lambrinos and Kleier 2003); in others (especially chemically defended lineages, such as dendrobatids: Savage 1968; Myers et al. 1991), all major activities (feeding, mating, etc.) occur by day (e.g., *Phylobates*—Duellman and Trueb 1986; *Crossodactylus, Hylodes, Megaelosia*—Jordão-Nogueira et al. 2006; *Taudactylus*—Liem and Hosmer 1973). Ontogenetic shifts in activity times (the strongest opportunity to examine correlates of diel cycles, as noted above) are widespread in "true toads" (Bufonidae and related families: Black and Black 1969; Minton 1972; Fitzgerald and Bider 1974; Taigen and Pough 1981; Duellman and Trueb 1986; Freeland and Kerin 1991; Sievert GA and Sievert LM 1993). Adults of most or all toad taxa are largely nocturnal, as is the general rule for anurans and presumably was ancestral for toads. However, a trend for immediately postmetamorphic individuals to be active by day rather than by night has been reported in a diverse array of toad taxa

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Address correspondence to R. Shine. E-mail: rics@bio.usyd.edu.au.

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(e.g., Black and Black 1969; Minton 1972; Fitzgerald and Bider 1974; Taigen and Pough 1981; Duellman and Trueb 1986; Sievert GA and Sievert LM 1993; Oishi et al. 2004). For example, cane toads (*Bufo marinus*) shift from diurnal to nocturnal activity when they attain body sizes of about 30 mm snout-urostyle length, a shift seen both in their invasive range within the Australian wet–dry tropics (Freeland and Kerin 1991) and in their native range in Central America (Zug GR and Zug PB 1979; Savage 2005).

At first sight, diurnal activity by metamorph toads is perplexing. High surface area to volume ratios mean that these tiny animals desiccate rapidly (Krakauer 1970; Seebacher and Alford 2002) and can overheat with a few minutes' exposure to direct sunlight (critical maximum temperature for premetamorphs is about 43 °C: Floyd 1984). Why, then, are small toads active mostly by day? Plausible answers include the following:

(1) Illumination level—perhaps metamorphs require high light levels for their eyes to function effectively in detecting prey and predators (metamorphs have smaller visual rods in their eyes than do adult conspecifics: Kinney and Fisher 1978);

(2) Vulnerability to predation—cannibalism is common, with larger juvenile toads specializing on smaller conspecifics as prey and relying on metamorph movement to trigger an attack (Hagman and Shine 2008; Pizzatto and Shine 2008);

(3) An endogenous day–night rhythm—some taxa are entrained to circadian rhythms, regardless of proximate conditions, and such entrainment may rely on specific neural capabilities that develop only gradually with age (Morgan 2004).

Other less likely possibilities include availability of insect prey (more common at dusk and into the night than by day: Pizzatto L, personal observation), thermal regimes (colder conditions may impair performance, but nocturnal temperatures are similar to diurnal temperatures for much of the wet season in our study area: Shine and Brown 2008), or hydric regimes (night rather than day is better for a small toad: Child, Phillips, and Shine 2008; Child, Phillips, Brown, and Shine 2008). We thus explored the role of the first 3 factors listed above, keeping the others constant, in influencing activity levels of metamorph toads, their response to disturbance, their feeding rates, and their probability of survival in the presence of a cannibalistic conspecific.

MATERIALS AND METHODS

Study species

The cane toad *B. marinus* (tentatively reallocated to the genus *Chaunus* by Frost et al. [2006] or *Rhinella* by Pramuk [2006]) is a large (to 24 cm snout-urostyle length, 2 kg) terrestrial anuran native to South and Central America but widely translocated elsewhere in attempts to control insect pests (Lever 2001). Cane toads were brought to Queensland in 1935 and are now abundant throughout most of tropical Australia (Urban et al. 2007). Their potent toxins are deadly for many Australian anuran predators (Phillips et al. 2003), and thus, the most important predators of cane toads in Australia may be other cane toads (Hagman and Shine 2008).

Desiccation risk restricts metamorph toads to the moist margins of natal ponds during the dry season (Child, Phillips, and Shine 2008; Child, Phillips, Brown, and Shine 2008). Metamorphs do not use refuges except to escape desiccating conditions near midday; the rest of the time (both by day and by night) they are found on the open muddy pond edges. Young toads are active and responsive by day (they move frequently and retreat if touched), whereas they are largely immobile at night (rarely moving and failing to respond to touch: Freeland and Kerin 1991; Pizzatto and Shine 2008). Risk of cannibalism offers a potential explanation for this nocturnal inactivity. Larger (juvenile) cane toads emerge from the surrounding vegetation at dusk and prey on their smaller brethren (metamorphs represented 67% of prey biomass ingested by larger toads captured around a single pond: Pizzatto and Shine 2008). Prey movement is necessary to elicit cannibalistic attack (Pizzatto and Shine 2008). Specialized toe-luring behavior by large juvenile toads attracts smaller conspecifics to within range of attack (Hagman and Shine 2008). Thus, nocturnal immobility may protect metamorph toads from cannibalism.

Experimental protocols

Toads were captured from waterbodies in the Adelaide River region and housed individually in plastic containers (5 × 10 × 7 cm), lined with wet soil and with ad libitum access to water, in a shaded outdoor area (and thus exposed to natural air temperatures and photoperiod). Food (termites and crickets) was provided twice weekly.

To explore the reasons for diurnal activity in metamorphs, we conducted experiments to test whether the major behaviors known to differ between night and day for metamorph toads (activity levels, antipredator responses, and foraging rates) result from some endogenous circadian rhythm or are direct responses to light levels or to the risk posed by a potentially cannibalistic conspecific. Similarly, is vulnerability to predation by larger toads dependent on circadian rhythm and/or light cues? Because thermal and hydric regimes do not seem to be plausible determinants of diel shifts in metamorph behaviors (see above), we simply held these variables constant, at levels that facilitated metamorph activity (Child, Phillips, and Shine 2008; Child, Phillips, Brown, and Shine 2008) rather than manipulating them. All experiments were run at 30 °C and 70% humidity. Diurnal trials were conducted between 1000 and 1200 h and between 1400 and 1600 h (thus avoiding the hot midday period when metamorphs are rarely active in nature), and nocturnal trials were conducted between 2000 and 2300 h. Each metamorph was used in only a single trial. All experiments, including those involving cannibalism, were approved by the Animal Ethics Committee of the University of Sydney (L04/5-2007/3/4515).

Is the level of spontaneous activity driven by light levels, by an endogenous circadian rhythm, and/or by predation risk?

We tested metamorphs under 8 sets of conditions, comprising an orthogonal combination of time of day (day vs. night), level of ambient illumination (artificial lights switched on vs. off), and predation risk (presence vs. absence of a larger [cannibal sized: see Hagman and Shine 2008; Pizzatto and Shine 2008] conspecific). Illumination was controlled by fluorescent lights, and windows were sealed to preclude ambient light. Both by day and by night, illumination levels within the room averaged 90 lux with the lights on and were undetectably low with the lights switched off (Minolta flash meter). Each testing arena consisted of a plastic bin (70 × 30 × 40 cm) lined with wet sand and soil, inclined at 5°, and with 1.5 L of water pooled on the lower side. Groups of 15 metamorphs (collected from nearby waterbodies within the preceding 48 h) were placed in each bin (total of 4 bins per experiment) and allowed to acclimate to the treatment conditions (e.g., day, dark, and no cannibal) for 1 h. To simulate predation risk but without inducing cannibalism (for ethical reasons), we fed larger toads to satiation (on crickets) 1 h prior to our trials. We then observed each focal metamorph toad (total of 50 individuals from the 4 bins) for a maximum of 2 min in turn to record if it moved spontaneously (i.e., changed location).

Is the diel rhythm of antipredator response driven by light levels, by an endogenous circadian rhythm, and/or by predation risk?

At the end of the 2-min observation period (above), each of the 50 observed metamorphs was lightly touched on the back with a twig. We recorded whether the animal moved away from the stimulus or remained immobile. This experiment was run in two 120-min blocks from 16 to 24 July 2007.

Is the diel rhythm of foraging affected by light levels, by an endogenous circadian rhythm, and/or by predation risk?

To answer this question, we conducted a new set of experiments but under the same conditions as the previous one. For these trials, we placed only a single metamorph in each container ($N = 10$ toads per treatment), added 10 worker termites (collected from nearby nests) to the container, and allowed the metamorph to forage for 5 min. At the end of the 5-min feeding period, we scored the number of termites remaining. To investigate whether a metamorph's foraging tactics are affected by the factors we manipulated, we measured feeding rates in small containers (15 × 10 × 7 cm: see above) as well as in larger containers (70 × 30 × 40 cm; $N = 10$ toads per treatment). In the larger bins, metamorphs had to move about to obtain prey items (we introduced the termites to the opposite half of the container to that of the metamorph, so that the young toads were forced to actively search for prey). In the smaller containers, a young toad could feed without engaging in locomotor activity (i.e., ambush predation). In the trials to determine if predation risk affected feeding rates, we added a well-fed adult toad to half of the larger containers. Adults and metamorphs were separated for the acclimatization period by a cardboard divider, which was removed immediately prior to the introduction of termites. The adult toads were given access to ad libitum food (crickets) 1 h prior to the trials to eliminate cannibalism. Experiments were run on 12 and 13 Feb 2007.

Does vulnerability to predation depend on light levels or an endogenous circadian rhythm?

We used the same day/night, dark/light treatments ($N = 15$ toads per treatment) as in the above experiments, but each metamorph was housed with one adult toad (the latter was food deprived for the preceding 6 h). We separated metamorphs and adults by a cardboard divider for the acclimation period. On removal of the divider, the trial was run for a further hour, after which we scored whether or not the metamorph had been consumed. The replicates within this experiment were run over a 30-h period on 15 and 16 Feb 2007.

What cues do metamorphs use to avoid larger conspecifics?

We used plastic gutters 2.3 m long and 10 cm high, lined with sand, and inclined to 5° to simulate the margin of a pond. The lower end contained water over a 27-cm length. Chemical and visual cues of larger (cannibal sized = 7.0–12 g : Hagman and Shine 2008; Pizzatto and Shine 2008) toads were presented in 4 ways:

(1) Visual plus chemical cues—a juvenile toad inside a 10 × 20–cm mesh box beside the pond edge;
(2) Visual but not chemical cues—a juvenile toad inside a transparent plastic box beside the pond edge;
(3) Chemical but not visual cues—3 g of toad scented unbleached toilet paper beside the pond edge; and
(4) Neither visual nor chemical cues—a juvenile toad inside an opaque (black) plastic box beside the pond edge.

To collect the toad scent, we housed adult toads (100–250 g) singly for 24 h in plastic containers (5 × 10 × 7 cm) lined with damp unbleached toilet paper. For the experimental trials, we released a single toad metamorph in the middle of the gutter's length after a 30-min acclimation period under a circular plastic container (7 cm in diameter) and filmed the gutter for 30 min. For each trial ($N = 15$ individuals per treatment), we scored 1) the duration of time each metamorph spent inside the pond; 2) the number of excursions to the pond; and 3) the maximal distance the young toad ventured into the pond. All trials were run during the day/light conditions described above, but in this set of trials, we maintained ambient humidity at 50% to stimulate toad movement to the pond edge. The experiment was run from 31 July to 6 August 2007.

Statistical analyses

We used 3-factor analysis of variance (ANOVA) to evaluate how a metamorph toad's food intake was affected by time of testing (day/night), illumination levels (light/dark), and the presence/absence of a potential cannibal in large and small arenas. We used logistic nominal regressions to test 1) effects of illumination levels (light/dark), time of testing (day/night), presence/absence of a potential cannibalistic conspecific, and bin identity as a random factor in a nonnested analysis on the occurrence of spontaneous movements (2 classes for the dependent variable: moved or not) by metamorph toads; 2) effects of illumination levels, time of testing, and presence/absence of a cannibal on the tendency of a metamorph toad to move away (2 classes for the dependent variable: moved or not) after it was touched; and 3) effects of light levels and time of testing on the incidence of cannibalism (2 classes for the dependent variable: metamorph eaten or not) (Sokal and Rohlf 1995). We used multivariate analysis of variance (MANOVA) and subsequent 2-factor ANOVAs to examine the effect of visual and chemical cues from a cannibalistic conspecific on the maximum distance the metamorphs ventured into the pond, the time spent in the pond, and the number of excursions to the pond. Our initial models always included main effects and all possible interactions of the tested variables, but when interactions were nonsignificant, they were excluded from the model and the analyses were run again.

RESULTS

Is the level of spontaneous activity affected by light levels, by an endogenous circadian rhythm, and/or by predation risk?

After correcting for bin effects ($\chi^2 = 12.58$, degrees of freedom [df] = 3, $P < 0.01$), the logistic regression showed that the probability of spontaneous movement by a metamorph toad was affected by ambient illumination levels (estimate = −0.398, standard error [SE] = 0.11, $\chi^2 = 12.33$, df = 1, $P < 0.001$) and by time of testing (day vs. night, estimate = 0.387, SE = 0.11, $\chi^2 = 13.16$, df = 1, $P < 0.001$). Metamorphs were more quiescent under darker conditions and at night. The presence of a potentially cannibalistic conspecific did not affect the tendency for spontaneous movement overall (estimate = −0.151, SE = 0.11, $\chi^2 = 1.94$, df = 1, $P = 0.164$), but the analysis revealed a significant interaction term between predator presence and time of testing (estimate = −0.212, SE = 0.11, $\chi^2 = 4.08$, df = 1, $P < 0.05$) as well as between time of testing and illumination level (estimate = −0.276, SE = 0.11, $\chi^2 = 6.33$, df = 1, $P < 0.02$). These interaction terms reflect a trend for both darkness and predator presence to inhibit spontaneous movement more by night than by day (Figure 1a).

Is the diel rhythm of antipredator response affected by light levels, by an endogenous circadian rhythm, and/or by predation risk?

We analyzed these data with logistic nominal regression. Whether or not the toad responded to our touch by moving away was affected by ambient light levels (χ^2 = 51.93, df = 1, $P < 0.0001$) but did not differ overall between trials conducted by day versus night (χ^2 = 0.47, df = 1, $P = 0.49$) nor by the presence of a predator-sized conspecific (χ^2 = 3.38, df = 1, $P = 0.07$). However, an interaction between these factors was significant. Brighter illumination increased toad responsiveness more by night than by day (χ^2 = 8.91, df = 1, $P < 0.003$) but did not modify the impact of predator presence on toad responsiveness (χ^2 = 0.47, df = 1, $P = 0.49$; see Figure 1b).

Is the diel rhythm of foraging affected by light levels, by an endogenous circadian rhythm, and/or by predation risk?

Food consumption was greater in well-lit conditions than in the dark both in small containers where metamorphs could forage without moving about and in larger bins where they had to travel to seize termites (Figures 1c and 2a). In both cases, the effect of light level on food intake was highly significant (in large bins, $F_{1,36} = 88.66$, $P < 0.0001$; in small containers, $F_{1,36} = 18.99$, $P < 0.0001$). Time of day did not significantly affect feeding rates either for metamorphs in small containers ($F_{1,36} = 1.28$, $P = 0.264$; see Figure 2a) or for those in larger bins ($F_{1,36} = 3.39$, $P = 0.08$).

Adding a large (and potentially cannibalistic) toad to each large enclosure significantly reduced food intake of metamorph toads ($F_{1,72} = 33.43$, $P < 0.0001$), especially in trials conducted in well-lit cages rather than in the dark (interaction, $F_{1,72} = 26.03$, $P < 0.0001$). As before, this interaction term reflects consistently low feeding rates in the dark, whereas predator presence could substantially reduce food intake in well-lit conditions that otherwise would have generated high rates of prey consumption (Figure 1c).

Does vulnerability to predation depend on light levels or an endogenous circadian rhythm?

Most metamorphs were consumed within the 1-h period if trials were held in darkened conditions at night, but vulnerability was lower under all other combinations (Figure 2b). Thus, the rate of cannibalism depended on light levels (χ^2 = 8.24, df = 1, $P < 0.001$) and time of day (χ^2 = 6.25, df = 1, $P < 0.02$), but the interaction between these factors was not significant (χ^2 = 0.012, df = 1, $P > 0.9$).

What cues do metamorphs use to avoid larger conspecifics?

MANOVA analysis revealed that the behavior of metamorph toads was affected by the presence of both visual (Hotelling–Lawley trace = 0.235, $F_{3,76} = 5.95$, $P = 0.0011$) and chemical (Hotelling–Lawley trace = 0.317, $F_{3,76} = 8.04$, $P = 0.0001$) cues from potentially cannibalistic conspecifics, as well as by an interaction between these 2 types of cues (Hotelling–Lawley trace = 0.173, $F_{3,76} = 4.39$, $P = 0.0066$). All 3 of these effects were evident on the maximum distance that metamorphs ventured into the pond (visual, $F_{1,78} = 16.63$, $P = 0.0001$; chemical, $F_{1,78} = 4.89$, $P < 0.03$; interaction, $F_{1,78} = 9.46$, $P < 0.003$): metamorphs entered further into the pond when they could not see or smell a larger toad. If both visual and scent cues were

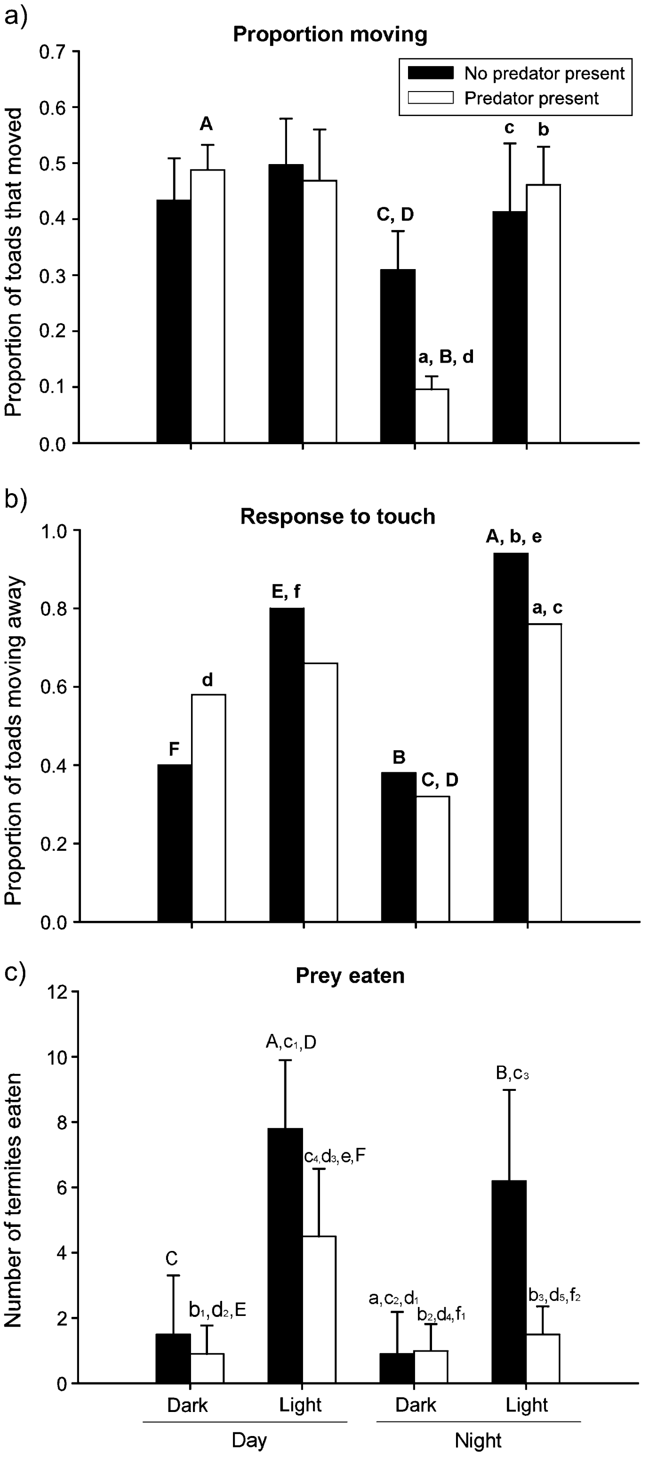

Figure 1
Effect of time of day, light level, and presence of a larger conspecific on (a) activity levels (effect sizes [{treatment1 - treatment2}/treatment1]: A,a = 4.1; B,b = 3.8; C,c = 0.3; D,d = 2.2), (b) response to disturbance (being touched on the back; effect sizes: A,a = 7.2; B,b = 1.1; C,c = 1.4; D,d = 0.8; E,e = 0.2; F,f = 1.0), and (c) feeding rates of metamorph cane toads housed in large containers (effect sizes: A,a = 7.7; B,b_1 = 5.9; B,b_2 = 5.2; B,b_3 = 3.1; C,c_1 = 4.2; C,c_2 = 0.7; C,c_3 = 3.1, C,c_4 = 2.0; D,d_1 = 7.7; D,d_2 = 7.7; D,d_3 = 0.7; D,d_4 = 6.8; D,d_5 = 4.2; E,e = 4.0; F,f_1 = 3.5; F,f_2 = 2.0). The graphs show mean values and associated SEs. Letters over the bars show significant differences between mean values.

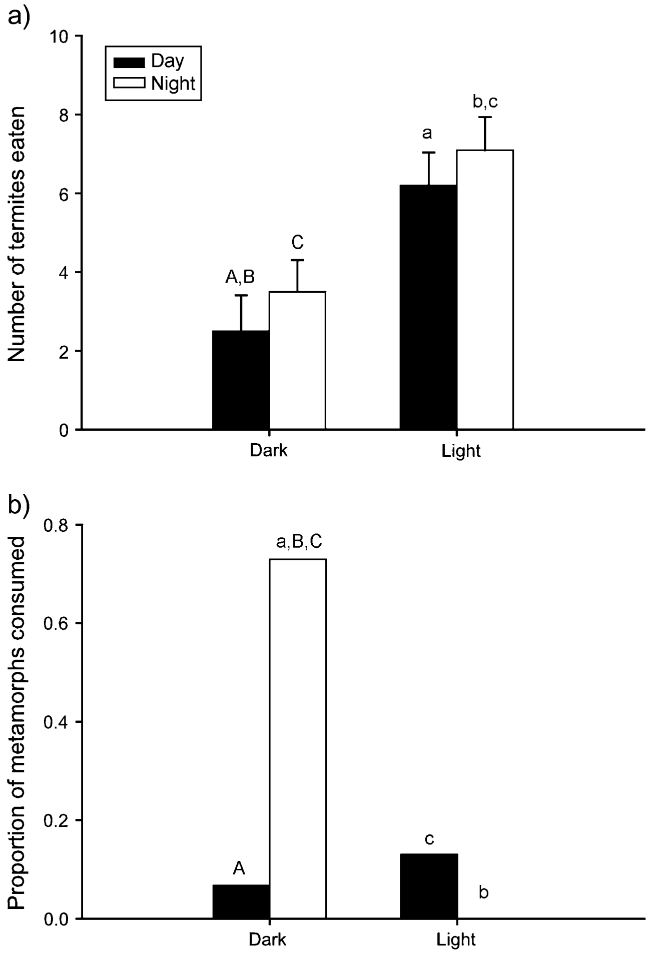

Figure 2
Effects of time of testing and illumination level on (a) feeding rates of metamorph cane toads housed in small containers (effect sizes: A,a = 1.5; B,b = 1.8; C,c = 1.0) and (b) the vulnerability of metamorph cane toads to predation by a larger conspecific (effect sizes: A,a = 9.9; B,b = 1.0; C,c = 4.6). The graphs show mean values and associated SEs. Letters over the bars show significant differences between mean values.

present, the young toads rarely ventured far into the pond (Figure 3a). The time spent in the pond was reduced by scent cues ($F_{1,78} = 12.64$, $P = 0.0006$) but neither by visual evidence of a larger toad's presence ($F_{1,78} = 1.15$, $P = 0.29$) nor by the interaction between visual and chemical cues ($F_{1,78} = 0.035$, $P = 0.85$; Figure 3b). Metamorphs made fewer visits to the pond if they could see a larger conspecific there ($F_{1,78} = 7.54$, $P = 0.0075$). Scent cues had no main effect in this respect ($F_{1,78} = 0.006$, $P = 0.94$) but reduced the number of pond visits if a larger toad was visible ($F_{1,78} = 8.11$, $P < 0.006$; Figure 3c).

DISCUSSION

Our laboratory trials clarify both the proximate and the ultimate factors affecting diel rhythms of activity in metamorph cane toads and the specific issues of what proximate cues drive diurnal behavior and avoidance of larger conspecifics.

Proximate causes of diel activity in metamorph toads

The major proximate cue influencing activity levels, defensive responses (tendency to move away from "attack"), and rates of food consumption of metamorph toads was illumination level.

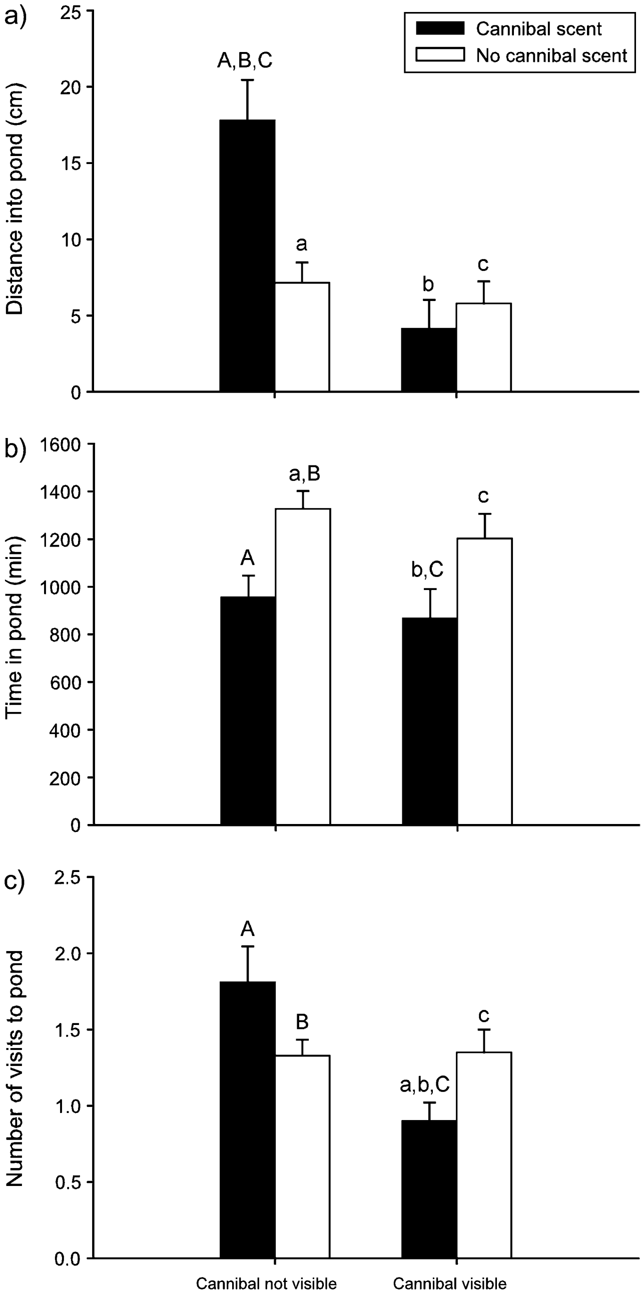

Figure 3
Effect of visual and scent cues from a larger (potentially cannibalistic) conspecific on the behavior of metamorph cane toads, *Bufo marinus*. (a) Maximum distance that a metamorph ventured into the pond (effect sizes: A,a = 1.5; B,b = 3.3; C,c = 2.1), (b) amount of time spent in the pond (effect sizes: A,a = 0.4; B,b = 0.5; C,c = 0.4), and (c) number of excursions to the pond (effect sizes: A,a = 1.0; B,b = 0.5; C,c = 0.5). The graphs show mean values and associated SEs. Letters over bars show significant differences between mean values.

The young toads responded directly to ambient light levels, increasing their activity in well-lit conditions and decreasing activity in the dark. The only obvious alternative cues that metamorphs might rely on to synchronize activity with daytime would be temperature (sometimes lower at night than by day), humidity, or some endogenous circadian clock. Temperature and humidity were held constant in our trials, precluding any role for these factors as stimuli for the diel shift in behavior. However, our results also reveal an endogenous rhythm in behavior: metamorphs tended to be more active, to respond more sensitively to disturbance, and to feed more rapidly by day than by night, regardless of other conditions. This pattern was manifested in significant interactions between light levels and time of testing for all variables that we measured. Although the day–night effect was weaker than the response to light levels in metamorph toads, our smaller data set on the larger (cannibalistic) toads suggests a strong endogenous rhythm. These cannibalistic toads largely refused to feed except in trials held under dark conditions and at night (Figure 2b).

Ultimate causes of diel activity in metamorph toads

Inferring ultimate (adaptive) functions for any given behavior is always difficult, even if the proximate mechanisms stimulating that behavior are well understood. For example, our work shows that ambient light levels exert a causal influence on metamorph activity, responsiveness, and feeding rates, but the adaptive significance of that causal link remains ambiguous. Young toads might benefit from activity under well-lit conditions either because 1) these conditions reduce the threat of cannibalism or because 2) these conditions enable successful foraging. In support of the latter hypothesis, the eyes of metamorph toads have smaller visual rods than those of conspecific adults (Kinney and Fisher 1978), and hence, the ontogenetic shift in activity times may have been driven by the young toads' inability to see clearly (and hence to forage effectively) under dim lighting. Equally, one could reverse cause-and-effect and interpret the relatively poor nocturnal effectiveness of metamorph visual systems as the mechanism by which natural selection has discouraged nocturnal activity.

In the face of such ambiguity, the most robust insights into the selective advantages of diurnal activity come from direct measures of costs and benefits, involving traits where the metamorphs' own behavior does not determine the outcome. This criterion is largely satisfied in our trials of vulnerability to cannibalism: metamorph behavior certainly influences vulnerability (see above and Hagman and Shine 2008; Pizzatto and Shine 2008), but in such a way as to generate the pattern opposite to that we see. That is, metamorph behaviors at night and in the dark are liable to make them less vulnerable (Figure 1), but in fact, these are the conditions where most metamorphs were consumed (Figure 2b). In combination with our field studies showing the same diel behavior shifts in metamorphs and the critical importance of prey movement in initiating cannibalistic attack (Pizzatto and Shine 2008), the current study provides strong support for the hypothesis that diurnal activity in metamorph toads lessens the risk from predatory conspecifics. Other advantages and disadvantages may be significant also, but this is the only function for which we have empirical evidence.

Even under low illumination levels, larger toads were reluctant to attack by day. Thus, metamorphs are likely to be safe from cannibalism even if they encounter a larger toad in its well-shaded diurnal retreat site. That invulnerability disappears with fading light levels at dusk, and immobility becomes the young toad's best defense. Why then do some metamorphs continue to move about at night (Freeland and Kerin 1991)? Can the advantages of nocturnal activity (greater availability of insect prey) sometimes outweigh the disadvantages (vulnerability to predation)? The answer may lie in seasonal and spatial shifts in the degree of danger posed by cannibalism. Nocturnal behavior may pose little risk to a metamorph under some circumstances, such as for the offspring from the season's first clutches (because there are no larger metamorphs around the pond margins to pose a threat) or during the wet season (when metamorphs rapidly disperse from the pond, again reducing encounter rates with larger conspecifics: Child, Phillips, Brown, and Shine 2008). In such a situation, we expect (and see) an additional facultative component to metamorph feeding rates and activity levels: the young toads detect and react to the presence of a larger conspecific. The end result is to amplify the diel shift evident even without a cannibal's presence.

How do metamorph toads identify potential cannibals?

Both visual and chemical cues from larger toads are used by metamorphs to detect the presence of a cannibal and thus modify their movements and habitat use. Avoidance of cannibalism may be an important influence on the ecology of metamorph toads, affecting their diel activity cycles as well as spatial ecology. That effect is partly facultative (i.e., the presence of a cannibalistic conspecific, or cues from that animal, modifies metamorph activity as well as habitat selection) but more importantly may have acted as a selective force to entrain diurnal behavior in the smallest (and hence most vulnerable) age class in the terrestrial phase of the toad's life cycle. In keeping with this hypothesis, Fitzgerald and Bider (1974) suggested that cannibalism might be an important reason why juvenile American toads *Anaxyrus americanus* are diurnal, whereas the adults are nocturnal. Future work could usefully examine whether similar ontogenetic shifts of activity times in other toad species are consistent with this hypothesis.

Visual and chemical cues are used by many animals to identify predators, and some species sensitively discriminate between cues of conspecifics versus heterospecifics (e.g., Mathis 2003). For example, juvenile Iberian rock lizards can discriminate among fecal chemicals deposited by conspecific juveniles, adult females, and males, and this discrimination influences the spatial distribution of juvenile lizards. In laboratory trials, juveniles moved away from chemical cues deposited by adult male conspecifics, thereby reducing the juvenile's vulnerability to cannibalistic attack (Moreira et al. 2008). Similarly, odonate larvae react strongly to chemical or visual cues from larger (and thus potentially cannibalistic) conspecific larvae (Ferris and Rudolf 2007).

Is cannibalism important in natural populations?

More generally, our results support the conclusion from previous work (e.g., Fox 1975; Polis 1981; Persson and Eklov 1995; Claessen et al. 2004; Rudolf 2007a, 2007b) that cannibalism can be a significant influence on individual behavior. The high frequency of cannibalistic interactions in many species may impose strong selection on smaller, more vulnerable individuals to behave in ways that reduce rates of encounter with larger conspecifics (Fox 1975; Rudolf 2007a). Evidence for this proposition comes from studies on a wide range of taxa. For example, young dragonfly larvae decrease their activity levels and switch to diurnal foraging when older larvae are present (van Burskirk 1992), very much like the facultative component of this behavior in cane toads. Small salamanders and lizards change their microhabitat use in the presence of larger conspecifics (Blomberg 1994; Rudolf 2006). Ontogenetic shifts in habitat in chameleons also may reflect avoidance of cannibalism (Karen-Rotem et al. 2006). Adult *Chameleo chameleon* are arboreal, whereas juveniles stay in grasslands. As in our own study system, the habitat selection of juvenile chameleons exposes them to unfavorable conditions (high temperatures, less shade, and less protection against predators) but protects them against cannibals (Karen-Rotem et al. 2006). One important feature of the cane toad system that we studied involves the reliance of anurans (especially small individuals) on moist substrates. This dependency may limit the ability of metamorphs to avoid larger conspecifics by shifting habitats (like the lizards and salamanders referenced above) rather than activity times. Indeed, during the wet season when the surrounding landscape is moist, cane toads emigrate from their natal ponds almost as soon as they metamorphose (Child, Phillips, Brown, and Shine 2008). This option is not available during the dry season, when the young toads must remain close to the ponds (Child, Phillips, Brown, and Shine 2008), and thus, shifting to diurnal activity may provide the only solution to avoiding cannibalistic conspecifics.

Ontogenetic shifts in the times and/or places of activity presumably are opposed by the many species-specific attributes that enable organisms to thrive under specific conditions (regardless of body size). Hence, ontogenetic divergence will evolve only when the costs of cannibalistic encounter outweigh whatever benefits keep older conspecifics within "preferred" habitats. Such costs will depend on many factors, but one critical issue may be the importance of predation risk from conspecifics as compared with other predator species. Invasive species such as the cane toads that we studied thus provide an extreme example because of their relative invulnerability to Australian predators (mostly unable to deal with the toad-specific toxins possessed by *B. marinus*: Phillips et al. 2003). If predation risk thus comes primarily from conspecifics rather than other predator species, metamorph cane toads may be under intense selection to adopt diurnal habits during the life-history phase when they are most vulnerable to cannibalism. The strong selection that cannibalism imposes on animal behavior may have major effects at both population and community levels. Recent studies reinforce the important role of intraspecific predation on population (Claessen et al. 2004; Persson et al. 2004; Andersson et al. 2007) and community structure dynamics, driving whole trophic cascades and shaping predator–prey relationships (Persson et al. 2003; Rudolf 2006, 2007a, 2007b).

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REFERENCES

Alanärä A, Burns MD, Metcalfe NB. 2001. Intraspecific resource partitioning in brown trout: the temporal distribution of foraging is determined by social rank. J Anim Ecol. 70:980–986.

Albrecht M, Gotelli NJ. 2001. Spatial and temporal niche partitioning in grassland ants. Oecologia. 126:134–141.

Andersson J, Byström P, Claessen D, Persson L, de Roos AM. 2007. Stabilization of population fluctuations due to cannibalism promotes resource polymorphism in fish. Am Nat. 169:820–829.

Angeli N, Pinel-Alloul B, Balvay G, Mènard I. 1995. Diel patterns of feeding and vertical migration in daphnids and diaptomids during the clear water phase in Lake Geneva (France). Hydrobiologia. 300/301:163–184.

Black JH, Black JN. 1969. Postmetamorphic basking aggregations of the boreal toad, *Bufo boreas boreas*. Can Field Nat. 83:155–156.

Blomberg SP. 1994. Body size, ecology and life history of the southern highland water skink. *Eulamprus tympanum* [PhD thesis]. [Sydney (Australia)]: University of Sydney.

Brattstrom BH. 1979. Amphibian temperature regulation studies in the field and the laboratory. Am Zool. 19:345–356.

Child T, Phillips BL, Brown GP, Shine R. Forthcoming. 2008. The spatial ecology of cane toads (*Bufo marinus*) in tropical Australia: why do metamorph cane toads stay near the water? Austral Ecol.

Child T, Phillips BL, Shine R. Forthcoming 2008. Abiotic and biotic influences on the dispersal behaviour of metamorph cane toads (*Bufo marinus*) in tropical Australia. J Exp Zool. 390A:215–224.

Claessen D, de Roos AM, Persson L. 2004. Population dynamic theory of size-dependent cannibalism. Proc R Soc Lond B Biol Sci. 271: 333–340.

Cogger HG. 1983. Reptiles and amphibians of Australia. Sydney (Australia): Reed Books.

Dahlgaard J, Hasson E, Loeschcke V. 2001. Behavioral differentiation in oviposition activity in *Drosophila buzzatii* from highland and lowland populations in Argentina: plasticity or thermal adaptation? Evolution. 55:738–747.

Dar S, Arizmendi MC, Valiente-Banuet A. 2006. Diurnal and nocturnal pollination of *Marginatocereus marginatus* (Pachycereeae: Cactaceae) in central Mexico. Ann Bot. 97:423–427.

Dolmen D. 1983a. Diel rhythms and microhabitat preference of the newts *Triturus vulgaris* and *T. cristatus* at the northern border of their distribution area. J Herpetol. 17:23–31.

Dolmen D. 1983b. Diel rhythms of *Triturus vulgaris* (L.) and *T. cristatus* (Laurenti) (Amphibia) in central Norway. Gunneria. 42:1–37.

Duellman WE, Trueb L. 1986. Biology of amphibians. Baltimore (MD): The Johns Hopkins University Press.

Dyer AC, Chittka L. 2004. Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study. J Comp Physiol A. 190: 105–114.

Ferris G, Rudolf VHW. 2007. Responses of larval dragonflies to conspecific and heterospecific predator cues. Ecol Entomol. 32: 283–288.

Fitzgerald GJ, Bider JR. 1974. Evidence for a relationship between age and activity in the toad *Bufo americanus*. Can Field Nat. 88:499–501.

Floyd RB. 1984. Variation in temperature preference with stage of development of *Bufo marinus* larvae. J Herpetol. 18: 153–158.

Fox LR. 1975. Cannibalism in natural populations. Annu Rev Ecol Syst. 6:87–106.

Freeland WJ, Kerin SH. 1991. Ontogenetic alteration of activity and habitat selection by *Bufo marinus*. Wildl Res. 18:431–443.

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, De Sá RO, Channing A, Wilkinson M, Donnellan SC, et al. 2006. The amphibian tree of life. Bull Am Mus Nat Hist. 297:1–371.

Hagman M, Shine R. 2008. Deceptive digits: the functional significance of toe-waving by cannibalistic cane toads (*Chaunus marinus*). Anim Behav. 75:123–131.

Halle S. 1993. Diel pattern of predation risk in microtine rodents. Oikos. 68:510–518.

Hansen EA, Closs GP. 2005. Diel activity and home range size in relation to food supply in a drift-feeding stream fish. Behav Ecol. 16: 640–648.

Heggenes J, Krog OMW, Lindis OR, Dokk JG, Bremnes T. 1993. Homeostatic behavioural responses in a changing environment: brown trout *(Salmo trutta)* become nocturnal during winter. J Anim Ecol. 62:295–308.

Hutchinson VH, Dupre RK. 1992. Thermoregulation. In: Feder ME, Burggren WW, editors. Environmental physiology of the amphibians. Chicago: University of Chicago Press. p. 206–249.

Jordão-Nogueira T, Vrcibradic D, Pontes JAL, Van Sluys M, Rocha CF. 2006. Natural history traits of *Crossodactylus aenus* (Anura, Leptodactylidae, Hylodinae) from Atlantic rainforest area in Rio de Janeiro, southeastern Brazil. South Am J Herpetol. 1:37–41.

Karen-Rotem T, Bouskila A, Geffen E. 2006. Ontogenetic habitat shift and risk of cannibalism in the common chameleon (*Chamaeleo chamaeleon*). Behav Ecol Sociobiol. 59:723–731.

Kinney MS, Fisher SK. 1978. Changes in length and disk shedding rate of *Xenopus* rod outer segments associated with metamorphosis. Proc R Soc Lond B Biol Sci. 201:169–177.

Kitchen AM, Gese EM, Schauster ER. 2000. Changes in coyote activity patterns due to reduced exposure to human persecution. Can J Zool. 78:853–857.

Kocárek P. 2001. Diurnal activity rhythms and niche differentiation in a carrion beetle assemblage (Coleoptera: Silphidae) in Opava, the Czech Republic. Biol Rhythm Res. 32:431–438.

Krakauer T. 1970. The invasion of the toads. Fla Nat. January 43: 12–14.

Kronfeld-Schor N, Dayan T. 2003. Partitioning of time as an ecological resource. Annu Rev Ecol Syst. 34:153–181.

Lambrinos JG, Kleier CC. 2003. Thermoregulation of juvenile Andean toads *Bufo spinulosus* at 4300 m. J Therm Biol. 28:15–19.

Lever C. 2001. The cane toad. The history and ecology of a successful colonist. Otley (UK): Westbury Academic and Scientific Publishing.

Liem DS, Hosmer W. 1973. Frogs of the genus *Taudactylus* with descriptions of two new species (Anura: Leptodactylidae). Mem Queensl Mus. 16:435–457.

Magnan P, Fitzgerald GJ. 1983. Ontogenetic changes in diel activity, food habits and spatial distribution of juvenile and adult creek chub, *Semotilus atromaculatus*. Environ Biol Fish. 11:301–307.

Marcelli M, Fusillo R, Boitani L. 2003. Sexual segregation in the activity patterns of European polecats (*Mustela putorius*). J Zool (Lond). 261:249–255.

Mathis A. 2003. Use of chemical cues in detection of conspecific predators and prey by newts, *Notophthalmus viridescens*. Chemoecology. 13:193–197.

Melamud A, Hagstrom S, Traboulsi EI. 2004. Color vision testing. Ophthal Gen. 25:159–187.

Metcalfe NB, Fraser NHC, Burns MD. 1998. State-dependent shifts between nocturnal and diurnal activity in salmon. Proc R Soc Lond B Biol Sci. 265:1503–1507.

Metcalfe NB, Fraser NHC, Burns MD. 1999. Food availability and the nocturnal vs. diurnal foraging trade-off in juvenile salmon. J Anim Ecol. 68:371–381.

Minton SA. 1972. Amphibians and reptiles of Indiana. Indianapolis (IN): Indiana Academy of Science.

Moreira PL, López P, Martín J. 2008. Discrimination of conspecific faecal chemicals and spatial decisions in juvenile Iberian rock lizards (*Lacerta monticola*). Acta Ethol. 11:26–33.

Morgan E. 2004. Ecological significance of biological clocks. Biol Rhythm Res. 35:2–12.

Myers CW, Paolillo A, Daly JW. Discovery of a defensively malodorous and nocturnal frog in the family Dendrobatidae: phylogenetic significance of a new genus and species from the Venezuelan Andes. Am Mus Novit. 3002:l–33.

Neuhaus P, Ruckstuhl KE. 2005. Sexual segregation in vertebrates. Cambridge (UK): Cambridge University Press.

Oishi T, Nagai K, Harada Y, Naruse M, Ohtani M, Kawano E, Tamotsu S. 2004. Circadian rhythms in amphibians and reptiles: ecological implications. Biol Rhythm Res. 35:105–120.

Persson L, Claessen D, De Ross AM, Biström P, Sjögren S, Svanbäck R, Wahlström E, Westman E. 2004. Cannibalism in a size structured population: energy extraction and control. Ecol Monogr. 74: 135–157.

Persson L, De Ross AM, Claessen D, Biström P, Lövgren J, Sjögren S, Svanbäck R, Wahlström E, Westman E. 2003. Gigantic cannibals driving a whole-lake trophic cascade. Proc Natl Acad Sci USA. 100: 4035–4039.

Persson L, Eklov P. 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology. 76:79–81.

Phillips BL, Brown GP, Shine R. 2003. Assessing the potential impact of cane toads on Australian snakes. Conserv Biol. 17: 1738–1747.

Pizzatto L, Shine R. Forthcoming 2008. The behavioral ecology of cannibalism in cane toads (*Bufo marinus*). Behav Ecol Sociobiol. .

Polis GA. 1981. The evolution and dynamics of intraspecific predation. Annu Rev Ecol Syst. 12:225–251.

Pramuk JB. 2006. Phylogeny of South American *Bufo* (Anura: Bufonidae) inferred from combined evidence. Zool J Linn Soc. 146: 407–452.

Rudolf VH. 2006. The influence of size-specific indirect interactions in predator-prey systems. Ecology. 87:362–371.

Rudolf VH. 2007a. Consequences of stage-structured predators: cannibalism, behavioral effects and trophic cascades. Ecology. 88: 2991–3003.

Rudolf VH. 2007b. The interaction of cannibalism and omnivory: consequences for community dynamics. Ecology. 88:2697–2705.

Sanchez-Vazquez FJ, Azzaydi M, Martinez S, Zamora S, Madrid JA. 1998. Annual rhythms of demand-feeding activity in sea bass: evidence of a seasonal phase inversion of the diel feeding pattern. Chronobiol Int. 15:607–622.

Savage JM. 1968. The dendrobatid frogs of Central America. Copeia. 1968:745–775.

Savage JM. 2005. The amphibians and reptiles of Costa Rica: a herpetofauna between two continents between two seas. Chicago: Chicago University Press.

Schoener TW. 1974. The compression hypothesis and temporal resource partitioning. Proc Natl Acad Sci USA. 71:4169–4172.

Schwaner T. 1989. A field study of behavioural thermoregulation in black tiger snakes (*Notechis ater niger*: Elapidae) on the Franklin Islands, South Australia. Herpetologica. 45:393–401.

Seebacher F, Alford RA. 2002. Shelter microhabitats determine body temperature and dehydration rates of a terrestrial amphibian (*Bufo marinus*). J Herpetol. 36:69–75.

Shine R. 1987. Intraspecific variation in thermoregulation, movements and habitat use by Australian blacksnakes, *Pseudechis porphyriacus* (Elapidae). J Herpetol. 21:165–177.

Shine R, Brown GP. 2008. Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet–dry tropics. Philos Trans R Soc Lond B Biol Sci. 363:363–373.

Sievert GA, Sievert LM. 1993. A field guide to the reptiles of Oklahoma. 2nd ed. Oklahoma (OK): Oklahoma Department of Wildlife Conservation.

Sokal RR, Rohlf FJ. 1995. Biometry: the principles and practices of statistics in biological research. 3rd ed. New York: WH Freeman and Company.

Taigen TL, Pough FH. 1981. Activity metabolism of the toad (*Bufo americanus*): ecological consequences of ontogenetic change. J Comp Physiol. 144:247–252.

Urban M, Phillips BL, Skelly DK, Shine R. 2007. The cane toad's (*Chaunus marinus*) increasing ability to invade Australia is revealed by a dynamically updated range model. Proc R Soc Lond B Biol Sci. 274:1413–1419.

van Burskirk J. 1992. Competition, cannibalism, and size class dominance in a dragonfly. Oikos. 65:455–464.

van Orsdol KG. 1984. Foraging behavior and hunting success in Queen Elizabeth National Park, Uganda. African J Ecol. 22:79–99.

Zug GR, Zug PB. 1979. The marine toad, *Bufo marinus*: a natural history resumé of native populations. Smithson Contrib Zool. 284: 1–58.