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A hot knot of toads: Aggregation provides thermal benefits to metamorphic Andean toads

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Abstract

We tested the thermal benefits of aggregation (“knotting”) for a high-elevation (>4300 m) population of metamorphic *Rhinella spinulosa*. Fewer knots were recorded when the sun was low on the horizon and aggregating toads dispersed under cloudy skies or when experimentally shaded. Body temperatures of sun-exposed toads averaged 2.3 °C higher when in knots than when solitary. Proximity to water and similarity in body size between aggregating and solitary toads suggest that grouping is not imposed by a need for reducing evaporative water loss. Aggregation apparently provides thermal benefits, which should translate into faster growth.

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1. Introduction

The formation of aggregations is widespread in animals and can have important social, ecological, and fitness consequences (Wells, 1977; Wilson, 1992; Caro, 1998; Krause and Ruxton, 2002). For some animals, aggregation is a behavior that buffers them from physiological challenges imposed by their abiotic environment (i.e. behavioral inertia; sensu Huey et al., 2003), such as low moisture availability (e.g. Cohen and Alford, 1996; Lancaster et al., 2006) or thermal extremes (e.g. Boersma, 1982; Kearney et al., 2001; Shah et al., 2003; Bloomstein et al., 2004; Schradin et al., 2006). For other animals, aggregation takes on a social role: finding mates, defending resources, or deterring predators or parasites (e.g. Wells, 1977; Arnold and Wassersug, 1978; Boersma, 1982; Sillen-Tullberg and Leimar, 1988; Watt et al., 1997; Wikelski, 1999; Krause and Ruxton, 2002; Spieler, 2003). Thus, the cues or selective

forces favoring aggregation vary along a continuum from strictly abiotic to primarily social (Lancaster et al., 2006).

The full range of this abiotic-to-social continuum is represented by the diversity of amphibian species that aggregate. Among anurans, aggregations are most commonly formed by individuals seeking mates (Wells, 1977; Sullivan et al., 1995; Stebbins and Cohen, 1997); however, both adults and larvae may form groups. In some cases the benefits accrued from aggregating are similar for both adult frogs and tadpoles. For example, tadpoles and metamorphic anurans are widely reported to raise their body temperature T_b by basking in groups when exposed to solar radiation (Hutchison and Dupré, 1992). Elevating T_b in this manner increases the rates of digestion, growth, fat deposition, and the ability to avoid predators (Seymour, 1972; Lillywhite et al., 1973; Carey, 1978; Brattstrom, 1979; Huey and Stevenson, 1979; Freed, 1980; Hutchison and Dupré, 1992; Rome et al., 1992)—consequences of thermal aggregations that should be closely linked to fitness. Accordingly, anurans that live in cool environments should experience strong selection for forming thermal aggregations.

One example of a cold-climate adapted frog is the Andean toad, *Rhinella [Bufo] spinulosa*. The geographic

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Fig. 1. A typical aggregation of recently metamorphosed Andean toads (*Rhinella spinulosa*) basking along the Río Cobres at Tocomar, Departamento Los Andes, Salta, Argentina (24.19309°S; 66.55339°W; 4323 m). Note the proximity of the toads to the water.

2. Methods

2.1. Study area

We studied a population of recently metamorphosed *R. spinulosa* in austral summer (mid January) 2006. The toads occupied a vega—a semi-saturated meadow of bunch grasses and sedges—along the Río Cobres at Tocomar (km marker 193 of Ruta Nacional 51, 38 km northwest of San Antonio de los Cobres), Departamento Los Andes, Salta, Argentina (24.19309°S; 66.55339°W; 4323 m). At this locality, dozens of small hot springs and seeps feed into the normally cool, shallow (5–50 cm deep) creek. The warmest springs reached 55–60 °C and were surrounded by blue-green algae and, more peripherally, filamentous green algal mats. Variation in water temperature was high and dependent on the temperature of, and distance from, the upwelling spring source(s), but in midstream typically ranged from ~10 °C in the morning (0900 h) to ~20 °C on sunny afternoons.

2.2. Characterizing the abiotic environment

We measured air temperature (T_a : 1 m, shaded bulb) and wind velocity (1 m, mean and maximum) every 1–3 h (depending on how rapidly conditions changed) with a Kestrel[®] 3000 pocket weather meter (www.kestrelwindmeters.com). Likewise, cloud cover was estimated at 1–3 h intervals. We recorded the temperature of the water (T_{water}) and substratum in deep shade (T_{shade}) and perpendicular to solar exposure on a dry mound of soil (T_{sun}) next to the river with a digital thermometer (Omega[®] 871A, type K thermocouple; Stamford, CT). Several of these measures (T_a , wind velocity, T_{shade} , T_{sun} , and cloud cover) were used to determine the times when thermoregulation could be most effective (i.e. highest environmental temperatures, lowest wind speed, and minimum cloud cover) and, thus, when it would be best to measure the T_b of aggregating and nonaggregating toads. We also assembled long-term climate records (Fig. 2) for San Antonio de los Cobres, Salta (24.1833°S; 66.3499°W; 3781 m), which is the closest weather station to the study site (38 km by road).

2.3. Role of time of day and solar radiation in propensity to aggregate

We define an aggregation (AKA: knot) as ≥ 5 individuals lying on, under, or adjacent to other metamorphs, with $\geq 25\%$ of the surface of their bodies touching at least one other frog in the knot. We counted the number of solitary toads and those found in knots over three census periods: morning (0930–0950 h, 19 January), mid-day (1330–1345 h, 18 January), and late afternoon (1535–1600 h, 18 January). The first two time periods were determined to be best for achieving high T_b : wind speed at 1 m above the ground surface ranged 0.4–4.0 m/s and the sky overhead was clear or nearly free ($\leq 10\%$) of clouds.

distribution of this species is extensive (albeit not entirely continuous): from Andean Perú south to Argentina, Bolivia, and Chile and extending into the valleys of northern Patagonia (Cei, 1979; Veloso and Navarro, 1988; Frost, 2007). This species also has an extensive elevational distribution: sea level to more than 5000 m (Gallardo, 1987; Veloso and Navarro, 1988; Lavilla and Cei, 2001). High-elevation environments pose a number of challenges for amphibians. These include low-density dry air, a high potential for heat gain via short-wave solar radiation by day, and high potential for heat loss via long-wave radiation at night (Swan, 1952; Navas, 1996, 1997). Collectively, these challenges translate into wide fluctuations in daily T_b . Adult Andean toads are well known for their behavioral and physiological adaptations to high elevation, and particularly to cold climates (Pearson and Bradford, 1976; Ruiz et al., 1989; Sinsch, 1989, 1990, 1991). More recently, the thermal biology of juveniles was studied at a high-elevation population in Chile (Lambrinos and Kleier, 2003). Like adults, juvenile toads achieve high T_b during the day by basking in direct solar radiation—despite the cool air temperatures—and seek shelter when it is cloudy (Lambrinos and Kleier, 2003; this study).

Here we describe the conditions under which recently metamorphosed *R. spinulosa* aggregate in a high-elevation environment. We hypothesized that juveniles, which unlike adults, are flat black dorsally (Fig. 1) form “knots” to achieve a thermal benefit. We show how both the tendency to aggregate and knot sizes vary with the time of day and prevailing weather conditions (sunny vs. cloudy) and how experimentally manipulating the availability of solar radiation changes the propensity of these toads to group. We discuss these findings in the context of their selective advantage for hastening growth.

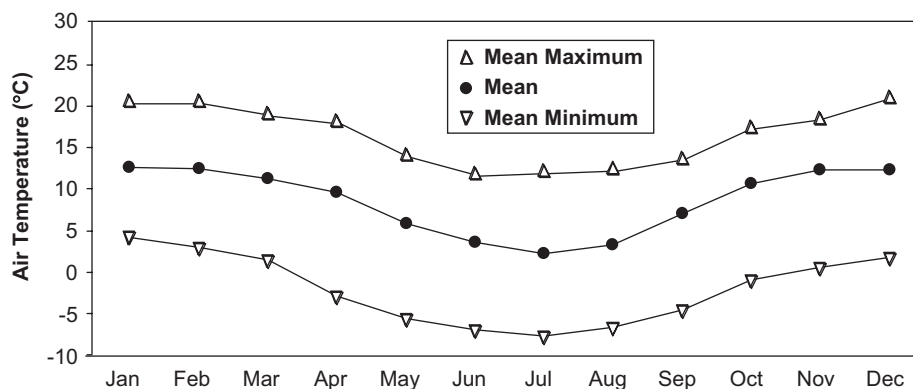


Fig. 2. Mean annual T_a at San Antonio de los Cobres, Salta (24.1833°S; 66.3499°W; 3781 m), which at 38 km (by road) is the closest weather station to the study site. Data courtesy of the Instituto Nacional de Tecnología Agropecuaria (INTA; 1927–1931).

The third time interval (1535–1600 h, 18 January) was a period of dense cloud cover, which allowed for comparisons between conditions varying in exposure to solar radiation. Counts of small knots were made by visual inspection at close range (standing or kneeling over the group), which did not require disturbing the aggregation. To count larger knots (>50), which typically consisted of 2–3 layers of toads, individuals from the upper layer(s) were removed from the vicinity of the knot after they were counted. We used a G^2 test of independence to compare the differences in proportions of individuals that were solitary or in knots under sunny and cloudy conditions and ANOVA to compare differences in the numbers of toads in knots under sunny or cloudy skies and as a function of time of day.

2.4. Body temperatures

We recorded the T_b of 95 solitary toads and 95 toads found in knots of varying sizes to compare the temperatures the animals achieved when conditions favored solar heating (as described above). Toads were handled by one of their limbs to minimize heat exchange with the investigator, and a 30-AWG (diameter = 0.5 mm, tip polished smooth) type-K thermocouple (Omega[®] 871A) was inserted 2–3 mm into the cloaca. Only T_b recorded within 5 s from the time the toad was captured were used in analyses. Measures of T_b were paired such that each T_b recorded for a toad in a knot was followed by a solitary toad found not more than 10 cm away to reduce measurement bias resulting from microhabitat selection or temporal variation in readings. Measurements were taken on 18 and 19 January from 1017 to 1320 h during times when solar radiation was unobscured (or nearly so) by cloud cover. Differences in T_b were tested with a two-tailed t -test.

Aside from thermoregulatory reasons, metamorphic toads may aggregate to lower their rates of evaporative water loss (EWL) by reducing their effective surface area (e.g. Cohen and Alford, 1996). If toads were grouping to reduce EWL, we expected to find a higher proportion of

smaller toads in knots because small-bodied individuals should benefit more from grouping owing to their higher surface-area-to-volume ratio. To address this non-exclusive alternative hypothesis, we compared the body sizes of 50 solitary toads to 50 toads found in knots. Because each toad weighed less than 0.5 g, we had to estimate their individual body mass (blotted dry) by weighing 50 of them in aggregate in a tared plastic bag, which was suspended from a 30-g Pesola[®] spring balance (www.pesola.ch/). We measured the snout–vent length (SVL; ± 0.5 mm) of the same 100 individuals with a transparent plastic ruler. Differences in SVL were tested with a two-tailed t -test.

2.5. Effect of shading on aggregation

We tested whether experimentally shading knots of toads changed the propensity of individuals to remain in the aggregations. We haphazardly selected 10 knots of toads on 19 January from 1100 to 1200 h (virtually no wind or clouds) and shaded five of them, with the other five serving as controls. Knots were shaded by standing near and casting the shadow of an opaque container lid (50 × 35 cm) ~1.5 m above the aggregation for 5 min. The shadow cast by the lid was at least five times the area of the largest knot shaded. To control for the potential effect of observer presence on aggregation propensity, the observer stood next to “control” knots for 5 min but did not shade them. We alternated which knots served as controls and which were shaded over the course of the experiments. The number of toads joining or leaving each knot was recorded during the trial, and after 5 min we counted the number of toads in the knot. Initial knot size was determined by adding the number of toads that left and/or subtracting the number of toads that joined the knot over the 5-min trial. Initial knot sizes ranged 9–47 individuals (mean = 20) and did not differ between the control and shaded treatments (ANOVA: $F_{1,8} = 0.098$; $P = 0.762$). To test for differences between control and shaded knots, we compared the number of toads that left or joined each aggregation using a two-sample, two-tailed t -test.

3. Results

3.1. Characterizing the abiotic environment

During our census periods in mid-January 2006, T_a ranged 4.5–19.0 °C (mean = 10.6 °C), T_{shade} 4.2–16.2 °C (mean = 10.9 °C), and T_{sun} 26.3–42.3 °C (mean = 32.3 °C). Environmental temperatures generally rose throughout the day until about 1600–1700h, after which cloud cover would substantially increase. Wind speed was generally low, with the mean ranging 0.4–4.3 m/s (grand mean-2.5 m/s) during census periods, and also generally increasing in the late afternoon, when on occasion gusts exceeded 8.5 m/s. Long-term climatic records show that T_a in this region is relatively cool, even during summer (Fig. 2), but recent research on Puna habitats (Piacentini et al., 2003) and our T_{sun} data indicate that the intense solar radiation at high elevation creates considerable thermal heterogeneity at the ground surface.

3.2. Role of time of day and solar radiation in propensity to aggregate

Our observations on propensity to aggregate are based on 3974 toads encountered over 183 observer minutes under sunny (108 min) and cloudy skies (75 min) over 2 d. We observed the first small knots (~5–6 individuals) forming by ~0930 h. Aggregation propensity varied relative to exposure to solar radiation. Under sunny skies, 56.4% toads (1693 of 3000) were found in knots of five or more, whereas only 22.4% (218 of 974) toads were found in knots of five or more under dense clouds ($G^2 = 358.488$; $df = 1$; $P < 0.0001$). We did not detect a statistical difference in knot size among times of day (ANOVA: $F_{2,78} = 1.634$; $P = 0.202$; Fig. 3A), likely because of the high variance within time periods (mean knot size \pm SE): morning (18.5 ± 3.7 toads/knot; $N = 20$), midday (27.0 ± 3.4 ; $N = 49$), and afternoon (18.2 ± 4.4 ; $N = 12$). A similarly large overlap in variance and the relatively low

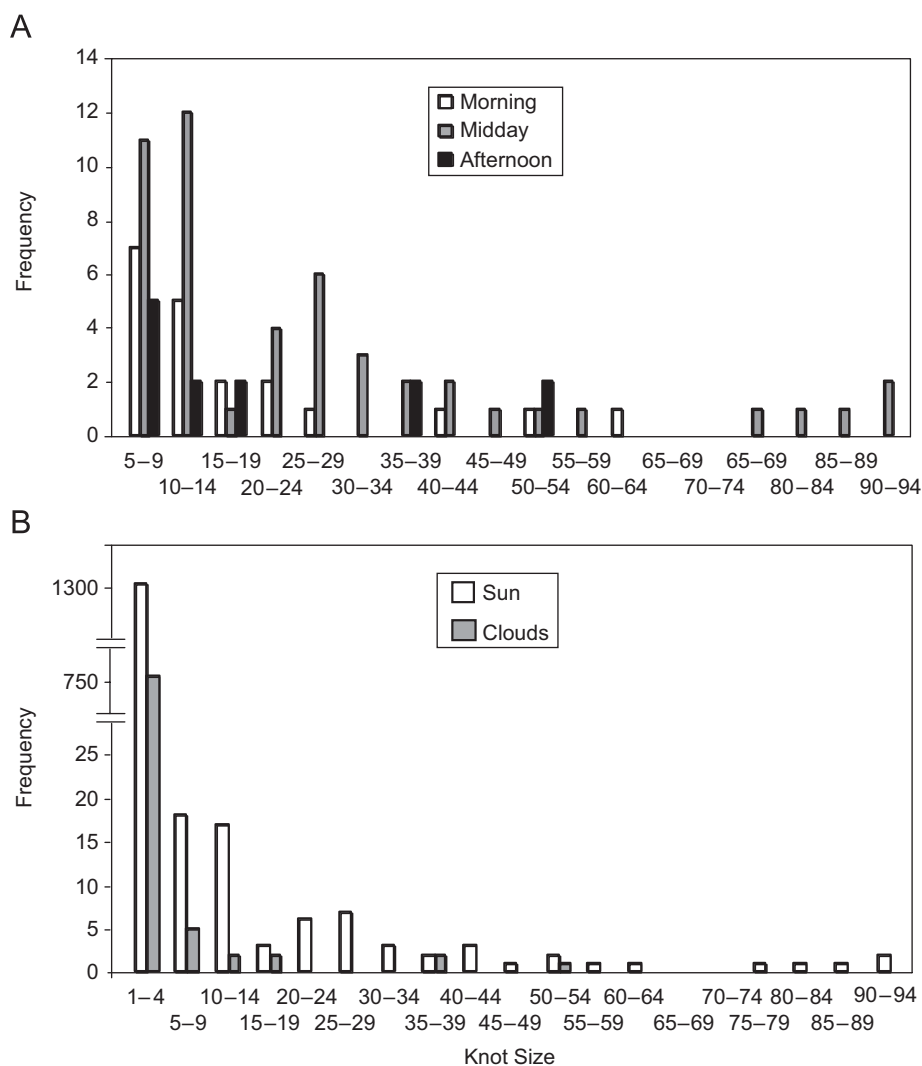


Fig. 3. Aggregation sizes of metamorphic *Rhinella spinulosa*. (A) As a function of census time (morning: 0930–0950; midday: 1330–1345; late afternoon: 1535–1600). Toads tended to form larger aggregations during midday when solar radiation was usually the most intense (i.e. fewer clouds). (B) As a function of solar radiation. Toads tended to aggregate more frequently and to form larger aggregations when exposed to solar radiation.

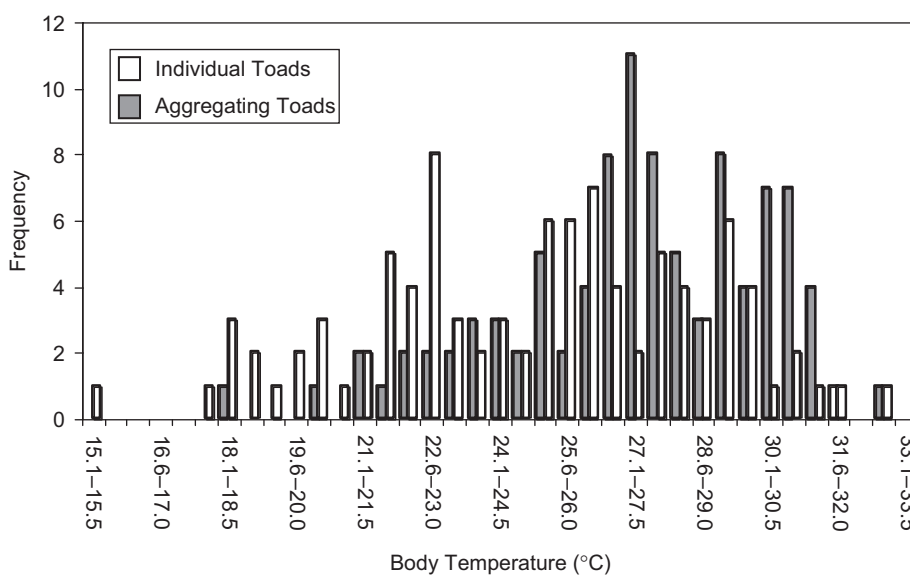


Fig. 4. T_b of metamorphic *Rhinella spinulosa* in aggregations ($N = 95$) and as solitary individuals ($N = 95$). T_b (mean \pm SD) of aggregating toads ($27.3 \pm 2.9^\circ\text{C}$) averaged 2.3°C higher than solitary toads ($25.0 \pm 3.8^\circ\text{C}$).

number of aggregating toads under cloudy skies likely prevented us from detecting a difference in knot size when it was sunny (24.5 ± 2.7 ; $N = 69$) compared to when it was cloudy (18.2 ± 4.4 ; $N = 12$) (ANOVA: $F_{1,79} = 0.914$; $P = 0.342$; Fig. 3B). However, under sunny skies, knots with ≥ 20 toads were common, whereas aggregations of this size were rarely encountered ($N = 3$) under cloudy skies (Fig. 3B).

3.3. Body size and temperatures

The body sizes of toads found in knots did not differ from those of solitary toads in either mass (0.42 vs. 0.44 g, respectively) or SVL (14.9 vs. 15.2 mm, respectively; $N = 50$; $t = 1.32$; $P = 0.195$). It is possible that our inability to detect differences in body size resulted from a lack of size variation in the population. To test this, we compared the SVLs of the 50 largest and 50 smallest toads (mean SVL = 16.0 vs. 14.1 mm, respectively) that we had measured. This analysis revealed that there was indeed detectable and significant body-size variation in our sample of the population ($N = 50$; $t = 24.00$; $P < 0.0001$). In addition, metamorphic toads were never observed more than 1 m from the water, and most were found on moist soil or vegetation within 20 cm of the stream (Fig. 1).

Over all sunny census periods, the mean T_b of toads in knots was 2.3°C higher than those of solitary toads (27.3 vs. 25.0°C , respectively; $N = 95$; $t = 6.09$; $P < 0.0001$; Fig. 4). The T_b of toads generally increased over the course of the day and there was a significant interaction between the time of day and whether the toad was in a knot, or not, on T_b (Fig. 5). In the morning, the mean T_b of toads in knots was 3.2°C higher than those of solitary toads ($N = 30$ per group; $t = 4.51$; $P = 0.0001$; Fig. 5). At midday, the thermal advantage for toads in knots

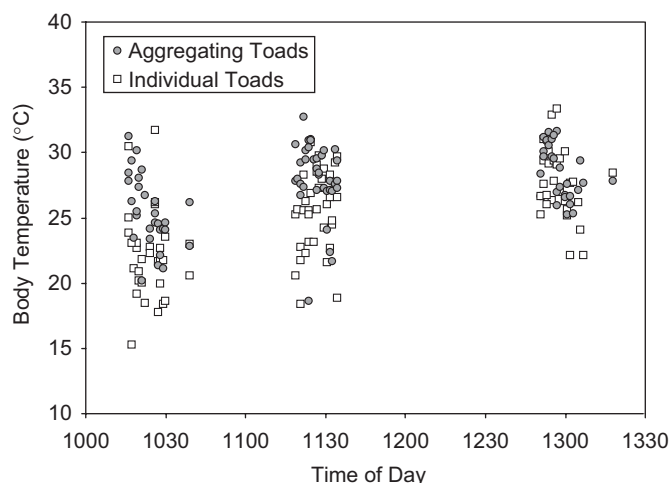


Fig. 5. T_b of aggregating and solitary metamorphic *Rhinella spinulosa* as a function of time of day. Toads achieved the greatest thermal advantage from aggregating in the morning ($T_b = +3.2^\circ\text{C}$ higher in knots) and midday ($+2.5^\circ\text{C}$), but this advantage diminished ($+0.9^\circ\text{C}$, and was no longer statistically significant) by early afternoon.

diminished to $+2.5^\circ\text{C}$ ($N = 36$ per group; $t = 4.08$; $P = 0.0003$; Fig. 5). Later in the afternoon, the difference between the knotting and solitary toads was both small ($+0.9^\circ\text{C}$ advantage for aggregating toads) and statistically indistinguishable ($N = 29$ per group; $t = 1.79$; $P = 0.083$; Fig. 5). Thus, the thermal advantage of knotting was most evident earlier in the day.

3.4. Effect of shading on aggregation

When experimentally shaded, the mean number of toads in knots declined by 30.7% (range = -9.1 to -90.0%), whereas the mean knot size in the unshaded controls

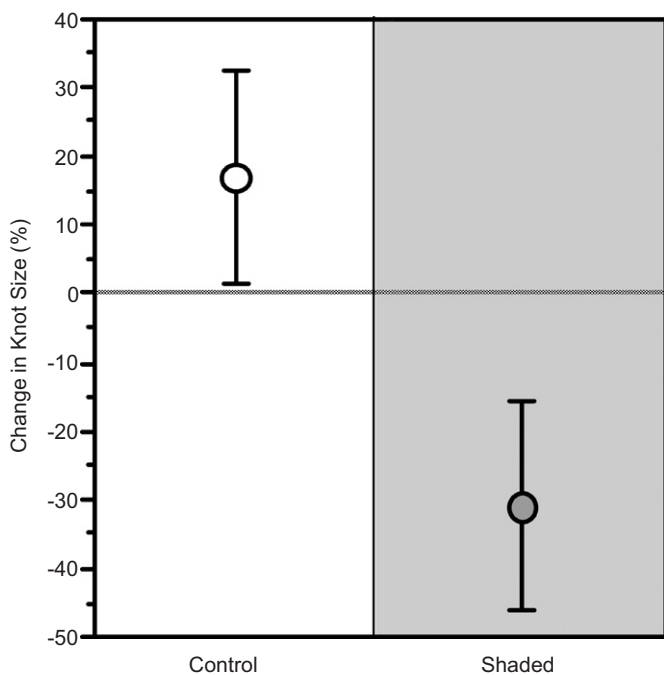


Fig. 6. Effect of 5-min experimental shading on propensity of metamorphic *Rhinella spinulosa* to aggregate. On average, shaded knots experienced a 30.7% reduction in toads, whereas the number of toads in control (unshaded) knots increase by 17.1%. Error bars are ± 1 SE ($N = 5$ knots/group).

actually increased by 17.1% (range = -22.7 to $+66.7\%$; $N = 5$ per group; $t = 2.235$; $P = 0.056$; Fig. 6).

4. Discussion

4.1. Aggregation cues

When individuals benefit from grouping by gaining heat either directly or indirectly from solar radiation, aggregating individuals typically disperse when solar radiation is obstructed by clouds or at night when the thermal advantage ceases (e.g. Griffiths, 1985). Our findings support this view for metamorphic Andean toads: (1) toads were more likely to aggregate during midday, when solar radiation was the most intense (Fig. 3A); and (2) aggregating toads dispersed each evening and when dense clouds appeared (Fig. 3B), or when knots were experimentally shaded (Fig. 6). Indeed, the largest knots we observed—estimated to have ~ 200 individuals—were only encountered under sunny skies. Unfortunately, attempts to accurately count these large aggregations failed. Our results pertaining to proclivity to bask during periods of solar exposure are similar to those found for a population of *R. spinulosa* at approximately the same elevation (4300 m) in northeastern Chile (Lambrinos and Kleier, 2003). But curiously, juvenile toads from that population do not aggregate (J.G. Lambrinos, pers. comm.), nor do juveniles in the population studied by Sinsch (1989, 1990, 1991) in central Perú at 3000–3600 m (U. Sinsch, pers.

comm.). We queried a number of amphibian researchers in Argentina also and, to our knowledge (and surprise), no other populations of this widespread toad have been reported to form diurnal aggregations as metamorphs when exposed to solar radiation. The lack of widespread aggregation in this species suggests that this behavior may be unique to this population and that there are locality-specific costs and benefits of aggregation that warrant further investigation.

4.2. Why are toads in knots hotter than solitary toads?

We have shown that metamorphic Andean toads found in knots achieve higher T_b than solitary toads (Fig. 4). Although our observations and experiments were not designed to identify the mechanism(s) underlying the thermal advantage of grouping, our studies inspire several nonexclusive yet testable hypotheses, which can be divided into mechanisms that increase heat gain and those that reduce heat loss. First, aggregation by these flat-black metamorphs (Fig. 1) should increase the rate of heat gain among group members (relative to heat loss via convection or evaporative cooling), such that the net exchange of heat with the environment is positive. Likewise, grouping reduces the surface area of individual toads, which should lower their rate of evaporative cooling, permitting toads in knots to achieve higher T_b . Both hypotheses could be tested by simultaneously measuring the rates of heating/cooling and EWL of solitary and aggregating toads or operative temperature (T_e) models thereof (e.g. Bartelt and Peterson, 2005). Third, aggregation may increase T_e by increasing T_a in the boundary layer of the knot. This could be tested by comparing the T_a at the boundary layer for solitary and aggregating toads or T_e models thereof. The relative importance of the factors that contribute to achieving high T_b are dependent on the mechanisms underlying heat gain and loss. These may vary by season, time of day, environmental conditions (e.g. solar angle, wind speed, relative humidity), and properties of individual toads or aggregations (e.g. size of knot, 3-D position in knot, mean wetness of toads in knot). For example, small knots will cool more quickly than large knots, especially when their boundary layer is disrupted by wind gusts. Likewise, “dry” knots (i.e. those formed by superficially dry toads) will be less prone to evaporative cooling than knots formed by wet toads.

4.3. Benefits of thermal aggregation in anurans

Both tadpoles and metamorphic frogs are widely reported to form diurnal aggregations and to receive thermal benefits from these groupings (Hutchison and Dupré, 1992; Stebbins and Cohen, 1997). In contrast to adults, which are primarily nocturnal, the larvae and metamorphs of many frog species, particularly high-elevation species, are primarily diurnal. Diurnal activity provides access to thermoregulatory opportunities, and

their associated benefits, which are not available to individuals that are active only at night. The small body size and flat-black color of metamorphic Andean toads should also increase their rate of heating and the amount of time (daily and seasonally) that they can sustain high T_b (Clusella Trullas et al., 2007).

Aggregating metamorphic Andean toads raised their T_b by an average of 2.3 °C (to 27.3 °C) when they had access to solar radiation (Fig. 4). This is similar to the T_b (27 °C) reported by Lillywhite et al. (1973) that maximized physiological performance for metamorphic *Anaxyrus* [*Bufo*] *boreas*. Because the *R. spinulosa* at Tocomar live in a relatively cool environment (Fig. 2), joining thermal aggregations would be advantageous because achieving and maintaining higher T_b increases locomotory performance, digestive capacity and efficiency, and rates of growth (Seymour, 1972; Lillywhite et al., 1973; Carey, 1978; Brattstrom, 1979; Huey and Stevenson, 1979; Freed, 1980; Hutchison and Dupré, 1992; Rome et al., 1992), which are reliable predictors of organismal fitness.

As an exercise in quantifying this thermal benefit for Andean toads, we estimated what the 2.3-°C increase in T_b achieved by the average toad in a knot would provide in terms of growth. We did this by combining estimates of growth rates of juvenile *A. boreas* maintained at one of three constant temperatures over a 7-week period (Lillywhite et al., 1973), our empirical data, and approximations of activity periods for metamorphic toads at our study site. We replotted data from Lillywhite et al. (1973; their Fig. 5) to obtain regression equations for temperature-dependent growth rates at 14, 20, and 27 °C ($r^2 = 0.99, 0.99, \text{ and } 0.57$, respectively). These equations were then used to interpolate or extrapolate growth rates for solitary (25.0 °C) or aggregating (27.3 °C) toads, respectively. We then fitted a second-order polynomial function ($r^2 = 1.00$) to the relationship between rearing temperature and the slopes for the growth rates from the Lillywhite et al. (1973) data set, and used that function to estimate growth rates (after converting from mm/week to mm/h) for solitary and aggregating toads. For purposes of this heuristic exercise, we made the following assumptions about metamorphic *R. spinulosa* from Tocomar: (1) growth starts at 15 mm SVL and the toads are not food limited; (2) knotting toads could achieve their thermal advantage for 4 h/d (1000–1500 h), which allows 1 h to leave the knot for non-thermoregulatory activities (e.g. foraging); (3) the T_b of toads during the activity season is 5 °C from early evening to the following morning (2000–0900 h) and 15 °C as toads warm up in the morning (0901–0959 h) and cool down in the early evening (1501–1999 h); (4) growth at 5 and 15 °C is negligible (Lillywhite et al., 1973); and (5) the activity season lasts 5 months (October–February). Based on these assumptions and estimates, our calculations indicated that juvenile Andean toads that participate in aggregations could achieve a 13.6% increase in growth rate over solitary toads (final SVL = 31.7 vs. 21.4 mm, respectively) during their first activity season. This size advantage

may prove important at the end of the season because larger individuals will have lower mass-specific metabolic rates and can accrue disproportionately larger fat reserves, which are critical for overwinter survival (Pinder et al., 1992). Of course, these estimates are meant to complement our observations and experiments, not to imply that there are solitary and knotting strategies or toad morphs in this population.

4.4. Alternative hypotheses for aggregation

Animals aggregate for a variety of reasons ranging from strictly physiological to primarily social (Krebs and Davies, 1993; Alcock, 2001; Krause and Ruxton, 2002; Lancaster et al., 2006). For the population of metamorphic *R. spinulosa* we studied, the reason appears to be physiological—specifically a thermal benefit—but several alternative hypotheses are worth considering. Grouping could also reduce EWL by lowering the surface area that individuals expose to the environment, as found for *R. marina* (Cohen and Alford, 1996). Yet the lack of a difference in body size between aggregating and individual metamorphic toads suggests that the animals were not grouping to reduce EWL. Although smaller toads might also have lower T_e independent of EWL, our assertion is further supported by the observation that the toads were always very close to the water (never >1 m and usually within 10–20 cm; Fig. 1). In most cases the substrata (sandy soil, grass, or sedges) on which aggregating toads congregated was moist, thereby permitting heating while maintaining water balance. Adult *R. spinulosa* (Sinsch, 1989) and other toad species (Seymour, 1972; Lillywhite et al., 1973) are more likely to bask in the sun when on moist substrata. Moreover, if these toads formed knots to reduce water loss, then we might also expect to find them thermoregulating in water rather than on land (e.g. Brattstrom, 1963). Indeed, our study site has many small hot springs and seeps, which would provide opportunities for toads to select high T_b while remaining hydrated. Tadpoles of this species, in fact, appear to use the hot springs for thermoregulation (pers. observ.). Although metamorphic toads were rarely found in the water, at least during our primarily diurnal observations, we did find a small number (<10) of dead metamorphs in a few of the hot springs. These toads apparently entangled themselves in the algae and succumbed to the lethal temperatures. Whether they had been using these hot springs for thermoregulatory purposes or were simply “passing through” is unknown, but the virtual absence of toads in these areas suggests that the hot springs are avoided because they are hazardous. This is likely because the high temperatures of the springs (exceeding the lethal limit tolerated by any amphibian) coupled with the high conductance of the metamorphs translate into a small margin for error (see also Licht and Brown, 1967). The strong scent of sulfur emitted from these springs suggests that they may pose a chemical threat as well.

In sum, body-water conservation was apparently less of a concern for these diurnally basking toads than was heat gain (contra Tracy et al., 1993). Yet our observations cannot rule out that terrestrial grouping provides a thermal benefit while simultaneously reducing water loss, because heat and water exchange are tightly coupled in amphibians (Tracy, 1976; Carey, 1978; Hutchison and Dupré, 1992; Tracy et al., 1993), and the relative importance of these processes is difficult to tease apart in a primarily observational study. Future studies should, therefore, use operative temperature models (Bakken and Gates, 1975; Bakken, 1976; Bartelt and Peterson, 2005) to examine the interactions of heat and water exchange between these toads and their unique environments (e.g. Bradford, 1984; Schwarzkopf and Alford, 1996).

Another alternative for grouping is to reduce the risk of parasitism (Hogue, 1972) or predation (Arnold and Wassersug, 1978; Sillen-Tullberg and Leimar, 1988; Krause and Ruxton, 2002; Spieler, 2003). There is no information about potential parasites of *R. spinulosa* at this site, but other frogs behaviorally select higher T_b (“fever”) when infected by pyrogenic microparasites (Kluger, 1977; Myhre et al., 1977). Hence, we cannot rule out the possibility that the aggregating Andean toads in this study were attempting to raise their T_b to fight infection. It was unlikely that predation posed a serious risk for the toads in our study. We observed no potential predators of the metamorphs in the vega and, given the elevation of the site (>4300 m), few vertebrate predators are likely to occur there. However, we did encounter a small number of eviscerated carcasses of adult *R. spinulosa* at Tocomar. Although we are uncertain of the identity of the predator(s), it seems likely that they were nocturnal, and therefore a carnivorous mammal, because adult Andean toads are primarily nocturnal (Pearson and Bradford, 1976; Sinsch, 1989; pers. observ.). It is possible that the same carnivore could be feeding on the metamorphic toads at night as well (consuming the entire animal), but, even if true, this would be irrelevant to our observations of strictly diurnal aggregations. Moreover, when experimentally shaded, toads in knots quickly dispersed (Fig. 6), which would not be expected if aggregation provided protection from parasites or predators.

Finally, it would be interesting to test whether metamorphic Andean toads also preferentially aggregate with siblings, as has been found in tadpoles of several other toad species (Blaustein and Walls, 1995; Waldman, 2001).

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