Using whole-group metabolic rate and behaviour to assess the energetics of courtship in red-sided garter snakes

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Reproductive effort is an important aspect of life history as reproductive success is arguably the most important component of fitness. Males tend to compete for access to females and, in the process, expend their energetic capital on mate searching, male–male competition and courtship rather than directly on offspring. Red-sided garter snakes, *Thamnophis sirtalis parietalis*, are an exceptional model for studying energetic costs of courtship and mating as they fast during the spring mating season, which segregates the cost of energy acquisition from the cost of courtship and mating. However, measuring an individual male's metabolic rate during courtship is complicated by the fact that male courtship behaviour in red-sided garter snakes is dependent on both the detection of a female sexual attractiveness pheromone and on facilitated courtship (i.e. vigorous courtship is only exhibited in the presence of other males). Thus, traditional techniques of placing a mask over the head of individuals would prevent male courtship behaviour, and single animals placed in a flow-through chamber would not yield ecologically realistic levels of courtship, which are only seen in the context of a mating aggregation in this species. Because of these difficulties, we placed groups of males in a flow-through metabolic chamber together with a single female whose respiratory gases were vented outside the chamber to yield a whole-group metabolic rate during competitive courtship. We also measured the standard metabolic rates (SMR) of the males individually for comparison with active metabolic rates. Conservative estimates of peak group metabolic rates during courtship are 10–20 times higher than resting group metabolic rate, which was 1.88 times higher than SMR. These measurements, coupled with the fact that these males are aphagous during the breeding, indicates that costs of courtship may be high for males and has implications for the male mating tactics in this system.
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Reproduction entails significant energetic expenditure that, in part, mediates life history trade-offs (Stearns, 1989, 1992). Likewise, the evolution of female–male sex roles, the coincident sex differences in longevity and the strength of sexual selection are attributed to differential allocation of energy beginning with gamete production and continuing through parental care (Barrett & Richardson, 2011; Bateman, 1948; Parker, 2014; Trivers, 1972). The challenges of courtship and direct male–male competition may require significant energetic expenditure and optimized performance, both of which have implications for reproductive fitness (Clark, 2012; Lailvaux & Irschick, 2006; Ryan, 1988). To minimize

these physiological costs and maximize fitness, males are expected to fine-tune courtship and competition effort relative to the potential for fitness payoffs (Barske, Schlinger, & Fusani, 2015; Byrne, 2008; Kahn, Dolstra, Jennions, & Backwell, 2013; deCarvalho, Watson, & Field, 2004). For example, male golden-collared manakins, *Manacus vitellinus*, increase the speed and frequency of courtship displays when a female is present, and even then limit these energetically expensive activities to short, but intense bursts (Barske et al., 2014, 2015). Likewise, the Australian Bibron's toadlet, *Pseudophryne bibronii*, strategically increase call intensity in response to the presence of females (Byrne, 2008).

Whether energetic investment is truly costly depends on a number of factors, most importantly, that resources are limited (Clark, 2012; Zera & Harshman, 2001; van Noordwijk & de Jong, 1986). For example, in some species, males abstain from foraging and feeding during the breeding season and rely solely on stored

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resources (garter snakes, *Thamnophis sirtalis*: O'Donnell, Shine, & Mason, 2004; southern elephant seals, *Mirounge leonine*: Galimberti, Sanvito, Braschi, & Boitani, 2007; northern elephant seals, *Mirounga angustirostris*: Sharick, Vazquez-Medina, Ortiz, & Crocker, 2015; emperor penguins, *Aptenodytes forsteri*: Le Maho, 1977; king penguins, *Aptenodytes patagonicus*: Schull et al., 2016). In such circumstances, any energetically demanding activity may be costly and restrict future opportunities for reproduction and survival (Crocker, Houser, & Webb, 2012; Yoccoz, Mysterud, Langvatn, & Stenseth, 2002). One way to assess the potential cost of courtship and male−male competition is to evaluate how males manage their metabolic rates or patterns of energy expenditure throughout contests or bouts of courtship (Clark, 2012; Lailvaux & Irschick, 2006). Male sierra dome spiders, *Neriene litigiosa*, for example, increase metabolic rate through sequential, stereotyped stages of combat where each act entails greater risk of injury, but also greater potential fitness payoffs (deCarvalho et al., 2004). However, studies like this are rare, partly because it is difficult to directly measure metabolic rate without interfering with courtship activities. In addition, the metabolic costs of courtship and contests are often confounded by other activities that require energetic capital (e.g. foraging and digestion, as pointed out by Galimberti et al., 2007). An ideal model system for the study of energetic costs of courtship is one where those costs are segregated from other activities. Such a system can be found in the red-sided garter snakes, *Thamnophis sirtalis parietalis*, of Manitoba, Canada.

Red-sided garter snakes are aphagous during the spring mating season (late April−May) and thus segregate the cost of energy acquisition for courtship and mating from costs associated with other activities (Friesen, Powers, Copenhaver, & Mason, 2015; O'Donnell, Ford, et al., 2004; O'Donnell, Shine, et al., 2004). As fasting through the breeding season makes the males energy-limited (Shine & Mason, 2005), they are likely to adjust courtship intensity to match the level of competition and potential fitness payoffs to minimize unnecessary energy expenditure (Joy & Crews, 1985). In this species, the sex ratio at spring emergence is strongly male biased. Several dozen males will converge upon a newly emerged female, attracted by her sex pheromone (Mason et al., 1989), forming 'mating balls' in which males court her and attempt copulation (Shine, Langkilde, Wall, & Mason, 2006; Shine et al., 2001). Male red-sided garter snakes that maintain persistent, high-intensity courtship at the moment just prior to copulation are more likely to mate (Shine, Langkilde, & Mason, 2004). Measuring an individual male red-sided garter snake's metabolic rate during courtship is difficult because mate assessment and male courtship behaviour are dependent on the detection of the female sexual attractiveness pheromone (LeMaster & Mason, 2001, 2002; Mason et al., 1989; O'Donnell, Ford, Shine, & Mason, 2004; Shine, Phillips, Waye, LeMaster, & Mason, 2003). Because of such difficulties, we placed groups of males in a flow-through metabolic chamber with a single female whose respiratory gases were vented outside the chamber to yield a whole-group metabolic rate during competitive courtship that we then used to estimate the average individual metabolic rate associated with courtship.

A key aim of our study was to test the utility of whole-group measurements of metabolic rate for assessing energetic investment towards courtship. A secondary aim was to evaluate predictions inspired by previous work on dome spiders (deCarvalho et al., 2004), namely that males should (1) increase metabolic rate over baseline in the presence of other males (i.e. adjust to competition), (2) further increase metabolic rate in the presence of a female and competition (i.e. adjust to potential fitness payoff) and (3) reach a maximum metabolic rate in response to competition (i.e. invest maximum effort at the critical period to increase chances of fitness payoff).

METHODS

Animal Collection

Thamnophis sirtalis parietalis ($N = 72\male$; $N = 12\female$) were collected by hand at a field site outside Inwood Manitoba, Canada during a single day in early May at the peak of the breeding season. These snakes were separated by sex and held in $1 \times 1 \times 1$ m seminatural enclosures located at the Chatfield Research Station, 16 km north of the Inwood field site. Water was provided ad libitum, but not food, as all the snakes are aphagous at this time (O'Donnell, Ford, et al., 2004; O'Donnell, Shine, et al., 2004). Males and females were weighed (± 0.01 g) and measured (snout-to-vent length, SVL, ± 1 mm) immediately prior to measurements of metabolic rate during courtship trials. Males were randomly assigned to groups (12 groups with six males in each). Although individuals within groups differed in mass, mass did not differ significantly between groups (see Results).

At the conclusion of our field experiments all male snakes used in the courtship trials were transported to George Fox University (Newberg, OR, U.S.A.) for measurement of standard metabolic rate (SMR). Each snake was kept separately in a 37.9 litre glass aquarium, in the laboratory under simulated spring conditions (artificial light 16:8 h light:dark cycle; ambient temperature 24:15 °C). A basking light (60 W incandescent) was provided at one end of the enclosure to allow daytime thermoregulation. Snakes were still aphagous during the SMR trials so were not fed, but water was provided ad libitum.

Metabolic Chamber and Respirometry Set-up

We used flow-through respirometry with an inlet tube and an outlet tube at opposite ends of the metabolic chamber. The metabolic chamber was constructed from a section of borosilicate glass tubing (112 mm outer diameter; 104 mm inner diameter; 78 cm long; Fig. 1). On each end, a 10 cm Schedule 40, PVC DWV hub with a female adaptor was tightly affixed and sealed with aquarium-grade silicone to make it airtight. Cap adaptors were threaded into the female adaptor at each end; the adaptors could be unthreaded to introduce animals to the chamber, but were airtight when fully threaded (threads were sealed with stopcock grease). A white acrylic floor was affixed within the glass tube using silicone caulking to provide a flat surface for courtship and reduce the total volume of the chamber to improve air sampling efficiency (effective volume of the chamber with the platform was 8700 cc; Bartholomew, Vleck, & Vleck, 1981). Inlet and outlet tubes used for air sampling were run through tightly fitting holes drilled into each cap, and the juncture between the tube and the holes were made airtight with silicone. A 100 CFM fan, built into the hub at the inlet end of the chamber, circulated the chamber air before reaching the outlet tube (a mesh screen prevented snakes from coming within 4 cm of the fan) to make the chamber time constant suitable for instantaneous corrections of metabolic rate during courtship trials (see below; Lighton, 2008). Temperature inside the chamber was monitored using a type-T (Cu−Cn) thermocouple and maintained at 29 ± 1 °C (29 °C is the preferred body temperature for these snakes; Aleksiuk, 1976) by heat lamps placed outside the chamber; output of the lamps was adjusted using a dimmer. The inlet air was scrubbed of water vapour using Drierite™ before being pushed through the chamber at 1000 ml/min (Sable Systems, Inc., Las Vegas, CA, U.S.A., MFS2 mass flow generator). Air from the outlet tube was subsampled with a Foxbox O_2−CO_2 analyser (Sable Systems, Inc.) at 500 ml/min. Water vapour produced by the snakes was scrubbed (Drierite™) prior to air flowing through the CO_2 sensor. Carbon dioxide and water vapour were scrubbed with soda