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Using Whole-Group Metabolic Rate and Behaviour to Assess the Energetics of Courtship in Red-Sided Garter Snakes

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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

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 ($N = 72$♂; $N = 12$♀) 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
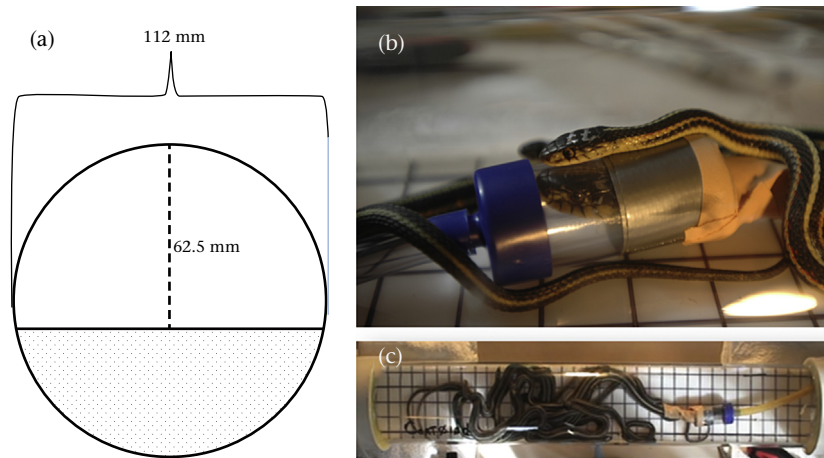
Figure 1. (a) Schematic of a cross-section through the metabolic chamber. (b) Male chin rubbing a masked female; the mask vented female respiratory gases from the chamber so they did not affect the measurement of male metabolic rate. (c) Overhead view of the metabolic chamber with five to six males intensely courting the female and performing tail-wrestling behaviour. The Tygon® tube venting the female's air from the chamber is visible on the right side of the chamber.

lime and Drierite™, respectively, prior to air flowing through the O_2 sensor. We report only the O_2 measurements here.

To prevent the female's respiratory gases from entering the main metabolic chamber, which would confound measurement of male metabolic rates, we fitted the female with a small, clear plastic mask. This mask was provided air through a short length of tubing that was vented outside the chamber (~12 cm long, 10 mm inner diameter, 14 mm outer diameter; Tygon®). The mask extended caudally from the female's head and was sealed by foam padding that lined the inside, rear edge of the mask, which also prevented chafing (Fig. 1b: picture of female with mask). Flexible adhesive tape further sealed and secured the mask in place (25 mm Nexcare™, 3M Co., Maplewood, MN, U.S.A.). The female was monitored closely and showed no signs of distress or discomfort during the trials. The effectiveness of the mask in isolating female respiratory gases was validated by comparing fractional oxygen concentration of air flowing through the chamber with and without the female present.

Data were acquired using Warthog LabHelper© data acquisition software and analysed using Warthog LabAnalyst© software (warthog.ucr.edu). Thus, the subsampled air, containing exhaled air from all six males simultaneously, was used to measure group metabolic rate (MR), which can be converted to an average rate for an individual male during courtship (i.e. MR/6). This individual MR calculated from a group MR is a conservative measurement of metabolic rate of maximum courtship intensity if not all males are courting to their fullest intensity.

Courtship Trials

A group of six males was placed in the chamber first and allowed to acclimate until all the males were at rest (i.e. 'loafing' and not moving around the chamber) and $\dot{V}_{O_2}$ was at equilibrium; this was the 'resting' metabolic rate for the group of males. Once the resting rate was acquired, the masked female was added to the chamber, the chamber was sealed and videorecorded (DCR-DVD505 Sony). Animals were monitored continuously (and videotaped) for at least 25 min to ensure that we obtained a metabolic measurement during maximum courtship activity. All trials were terminated after copulation occurred or after 45 min to avoid overheating, whichever came first. This procedure was repeated with 12 groups of males. The timing of activities was synchronized in LabHelper with

markers on the metabolic trace. Specific markers on the metabolic trace were also used in conjunction with our hand-recorded notes and video analysis to determine minimum and maximum courtship intensity (see below).

Courtship behaviour of garter snakes is straightforward to recognize and score (Blanchard & Blanchard, 1941; Crews et al., 1984; Noble, 1937). Male courtship behaviour is composed of a stereotyped sequence of behaviours. These behaviours are initiated when the male detects the female sex pheromone with the vomeronasal organs by tongue flicking (Kubie, Vagvolgyi, & Halpern, 1978; Mason et al., 1989). Upon encountering an attractive female the male's tongue-flicking rate increases. The male then positions his body in line with the female. The male presses his chin against the female's skin and moves along the length her body, sometimes reversing direction until his head is behind hers. Courtship is the only context in which chin-rubbing behaviour is exhibited. The male then displays tail-to-head contractile waves along the length of his body against her body (i.e. caudocephalic waves). The male then wraps his tail around the female, attempting to lift her tail and manoeuvre towards cloacal apposition. When other males are also engaged with the same female, male tail wrestling becomes corybantic. If the female is sexually receptive she will lift her tail and gape her cloaca, which allows a male to intromit one of his two hemipenes. Because this behaviour is stereotyped and increscent, it is possible to assign males a score on a scale from 0 to 5 (in increments of 1) with 0 indicating the male did not approach the female and 5 being an attempted intromission (see Table 1; Moore, Lemaster, & Mason, 2000). Video was scored by an observer who was blind to metabolic traces and trained to identify courtship scores based on a formalized ethogram of courtship behaviour described above. The observer noted the time and number of males engaged in each type of activity for each trial and these were compared with the first-hand notes recorded during the trials to corroborate the association of metabolic rates with activity.

We qualitatively described the patterns of group activity by tallying the number of males that engaged each courtship activity for the duration of the trial. A group courtship score (GCS) was calculated as the product of the courtship score based on the ethogram in Table 1 (levels 1–4, excluding level 5 for mating), and the number of males engaged at that level of courtship. For example, if all six males in the chamber received a courtship score of 4, then the GCS would be 24 (6 males × 4 = 24), which was the

Table 1
Ethogram of male garter snake courtship behaviour (modified from Moore et al., 2000)

Courtship score	Description of male behaviour
0	No reproductive behaviours exhibited
1	Investigates female
2	Chin-rubs female, rapidly tongue-flicks female's skin
3	Body is aligned with female
4	Actively tail-searches female, attempts cloacal apposition; possible caudocephalic waves
5	Copulation or attempted intromission

Scores ≥ 2 are only displayed in a reproductive context (Crews & Moore, 1986; Moore et al., 2000). Resting males are defined as males scoring 0 or 1. Minimum courtship was defined as at least four of six males engaged in behaviour scored as level 2–3. Maximum courtship was defined as at least four of six males engaged in behaviour scored as level 4 and just prior to level 5 (mating or attempted intromission). Average courtship metabolic rate was defined as the average metabolic rate over periods where any males engaged in courtship that scored ≥ 2, and is thus inherently conservative.

maximum possible score. If four males received a courtship score of 1 and two males received a score of 3, then the GCS would be 10 ((4 males × 1) + (2 males × 3) = 10). There are certainly ambiguities generated by this score, such as where one male receiving a score of 6 and five males receiving a score of 0 would yield the same group score as all six males receiving a score of 1. However, the high GCS (e.g. >14) can only be reached with most males engaging in robust courtship activity of ≥ 3 and it takes into account variation of individual courtship activity, unlike other 'composite' measure developed in this system (i.e. Joy & Crews, 1985). Furthermore, in practice, males seemed to match behaviours, such that most males engaged in similar activities. We then calculated the mean duration of bouts of activity in each trial. For example, if five males were courting at level 3 for 3 min, and then two males left the group or changed their courtship activity (thus changing the group courtship score), we would then calculate the duration of this level of group activity until it changed. In theory, this could be a tedious process, with ever more finely divided periods, but as described above, the males seemed to cycle through bouts of courtship nearly in unison.

Analysis of Courtship Metabolic Rates

We report four measures of courtship MR, all of which were divided by six to estimate the metabolic rate of an average individual male during courtship: (1) resting metabolic rate (RMR) is the metabolic rate when no males are courting and is equivalent to scores of 0–1 on an established courtship ethogram (see Table 1; Crews et al., 1984; Moore et al., 2000); (2) minimum courtship MR is the average metabolic rate recorded during periods in a courtship trial when at least four males were courting; (3) average courtship MR is the average rate over the entire course of a courtship trial; (4) maximum courtship MR is when at least four males engaged in the highest level of courtship including caudocephalic waves and tail-searching behaviour. Because the number of courting males and courtship intensity was not constant for extended periods, we estimated MR during courtship trials using an instantaneous correction (Bartholomew et al., 1981; Lighton, 2008). We compared these four measures of courtship MR with a fifth measurement, the standard metabolic rate (SMR) described below.

Measurement of Male Standard Metabolic Rates (SMR)

All males ($N = 72$) used in the courtship experiment were transported to George Fox University for more highly controlled

and precise measurement of standard metabolic rates (SMR) than would be possible under field conditions. Standard metabolic rate (SMR) represents the minimum metabolic rate needed to sustain life at a specified temperature (usually preferred body temperature) in organisms that are resting in a postabsorptive state and in darkened conditions (McNab, 2002). All SMR measurements were made at 29 °C and within 2 weeks of courtship trials while males were still in the posthibernation fasting phase of their annual cycle (males remain near emergence sites for 2−6 weeks while in a fasting state; O'Donnell, Ford, et al., 2004; O'Donnell, Shine, et al., 2004). To measure SMR, individual snakes were sealed in airtight, cylindrical aluminium metabolic chambers (diameter: 12 cm, height: 12 cm, volume: 225 cc), and placed in an environmental chamber (Percival I-35VL) to precisely control measurement temperature. After a 60 min equilibration period, SMR was measured using positive-pressure, open-flow respirometry, and analysed as described above for RMR except that chamber flow-rate was controlled at 300−350 ml/min. Only trials where $\dot{V}_{O_2}$ was at equilibrium for a minimum of 10 min were used for analysis.

Ethical Note

Animals used in this study were unharmed. This research adhered to the ASAB/ABS Guidelines for the use of animals in research and was conducted under Oregon State University Institutional Animal Care and Use Committee protocol no. ACUP-3738. Animals were collected under a Manitoba Conservation Collection and Use permit no. WB12405. The females used in this study were released at point of capture with 24 h of the courtship trials. Although four males were excluded from SMR measurements because of poor body condition, these animals regained body condition within 2 weeks and all 72 of the males that were transported to George Fox University were returned and released healthy at point of a capture the following spring per a requirement of our WB12405 permit.

Statistical Analysis

We tested for the effect of individual male mass on standard metabolic rate using ANCOVA with group as a factor and male mass as a covariate to assess whether the groups of males we used in the courtship trials differed in SMR. When we found that these groups did not differ in SMR, we then used simple linear regression to plot SMR as a function of male mass. Standard, resting, minimum courtship, average courtship and maximum courtship metabolic rate were log transformed to improve homogeneity of variance and normality among groups. We used the within-group mean of individual SMR values to compare SMR data to our group measurements of metabolic rates obtained in the courtship trials for analysis. We tested for differences between measures of metabolic rate using generalized linear mixed models (GLMM; normal distribution, identity link function) with group fitted as a random effect and MR 'type' as a fixed effect. Average group body mass was dropped as a covariate in this model for three reasons: (1) average body mass did not differ between groups; (2) average body mass was not a significant predictor when included in the GLMM; and (3) excluding average body mass improved the fit of the model (Akaike's information criterion corrected for small sample size, AICc: −34.4 to −42.0). Pairwise contrasts of activity levels were generated using SPSS v.22 statistical software (IBM, Armonk, NY, U.S.A.). We calculated means and standard deviations for GCS and activity durations in MS Excel. With such small sample sizes and a larger number of factors, we restricted ourselves to using descriptive statistics rather than significance testing for the behavioural data.

RESULTS

SMR

Of the 72 males used in the courtship trials, four were excluded from $SMR_{29°C}$ measurements because of their poor condition after transport. For the remaining 68 males, SMR was positively correlated with male mass (ANCOVA: mass * courtship group interaction was dropped as $P = 0.611$; ANCOVA: $R = 0.707$; courtship group: $F_{11, 55} = 1.625$, $P = 0.117$; mass: $F_{1, 55} = 41.419$, $P < 0.001$; see Fig. 2). There were no significant differences in individual masses among groups ($P = 0.099$).

Active MR

Differences in metabolic rate were positively related to courtship activity levels (GLMM: AICc = -42.028; $F_{4, 53} = 180.709$, $P < 0.0001$) and all pairwise contrasts between activity levels were significant ($P < 0.001$; Fig. 3). Raw values for oxygen consumption were as follows: standard MR (mean = 0.044 ml of O_2/min; 95% confidence interval, CI: 0.041−0.047); resting MR (mean = 0.08 ml of O_2/min; 95% CI: 0.07−0.10); minimum courtship MR (mean = 0.15 ml of O_2/min; 95% CI: 0.12−0.18); average courtship MR (mean = 0.27 ml of O_2/min; 95% CI: 0.21−0.33); maximum courtship MR (mean = 1.49 ml of O_2/min; 95% CI: 1.03−1.95).

Factorial Increase in MR

Factorial scope is the ratio between the metabolic rate of maximum activity and resting rate and it is useful for gauging the magnitude of increased metabolic rates associated with a particular activity (e.g. Moberly, 1968; Peterson, Nagy, & Diamond, 1990; Ryan, 1988). We calculated two measures of factorial scope: average courtship MR/resting MR = 3.2; maximum courtship MR/resting MR = 17.7. We also calculated the factorial increase of each measure of metabolic rate to gauge the relative increase in energetic investment: RMR/SMR = 1.88; minimum courtship/RMR = 1.84; average courtship/minimum courtship = 1.77; maximum courtship/average courtship = 5.49. Thus, the largest factorial increase in metabolic rate was associated with levels of courtship activity that occur just prior to mating (i.e. tail searching and caudocephalic waves). We converted metabolic rate into a measure of work (Joules: $kg \times m^2/s^2$) assuming an enthalpy of combustion for O_2 of 20.1 J/ml (Koteja, 1996).

Courtship Behaviour

Average group courtship score (GCS) throughout each trial and averaged across all trials ($\pm$SD) was 9.41 $\pm$ 2.07, which corresponds with our average courtship metabolic rate data. On average ($\pm$SD), males maintained a GCS of $\geq$10 for 37.32 $\pm$ 6.02% of a trial and maintained a GCS $\geq$15 for 25.70 $\pm$ 11.47% of the trial. Periods of GCS $\geq$15 averaged 2.25 $\pm$ 1.09 min in duration and occurred 4.2 $\pm$ 2.05 times per trial. Mean ($\pm$SD) trial length was 36.89 $\pm$ 6.02 min, so on average 9.48 $\pm$ 4.78 min were spent at GCS $\geq$15. These periods of GCS $\geq$15 correspond to when maximum MR measurements were taken. The highest GCS reached within a trial ranged from 10 to 24 across all trials and averaged 18.75 $\pm$ 4.14. Matings ($N = 3$ trials) always coincided with GCS $\geq$15 (GCS: 17, 20, 24). Median within-trial GCS coincident with female movements was 15.5 (range of within-trial averages, 10−18). This indicates that female movement was associated with GCS >15. Periods of peak group courtship activity were interspersed with lower levels of activity (mean $\pm$ SD GCS = 5.91 $\pm$ 1.10), which averaged 2.99 $\pm$ 1.81 min in duration.

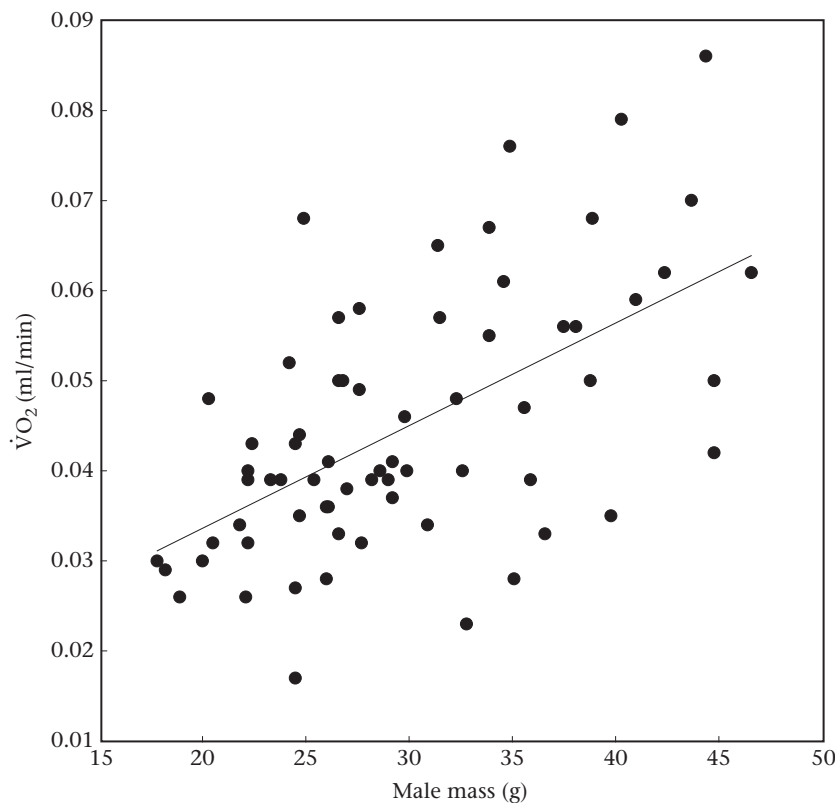**Figure 2.** Regression of individual standard metabolic rate on body mass for each male in the study at 29 °C (mass$^{0.84\pm1.4}$; $R^2 = 0.3871$, $P < 0.0001$).
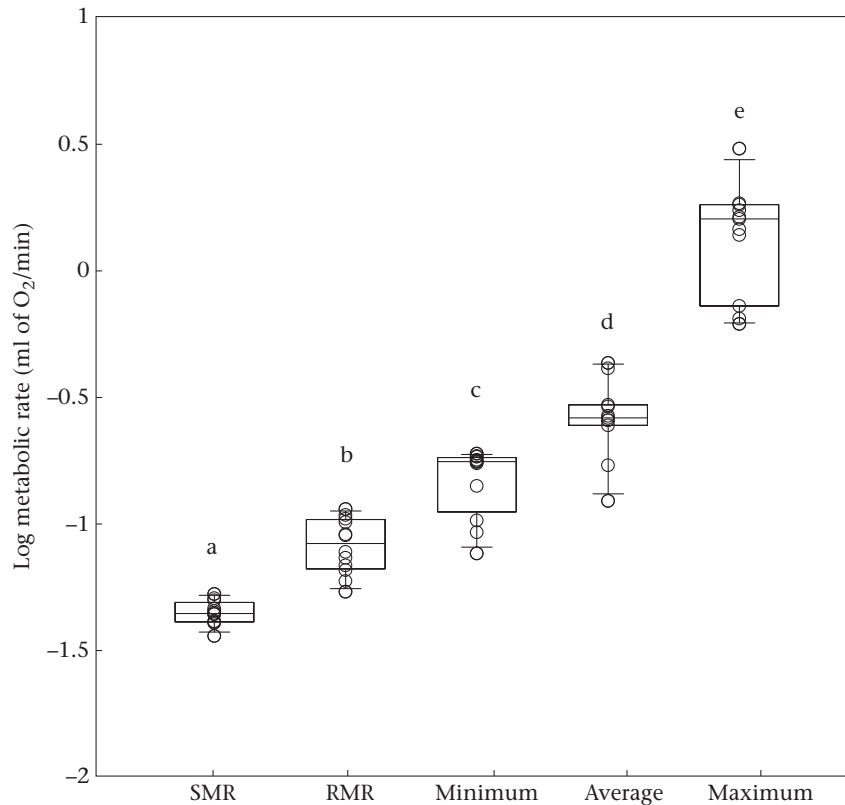
Figure 3. Individual metabolic rate as a function of activity level estimated by dividing the whole-group metabolic rate by the number of males in the group, which was always six (i.e. log (ml of O₂ per min per 6 males)). Standard metabolic rate (SMR) is the average of individually measured oxygen consumption in the dark at 29 °C; resting metabolic rate (RMR) was recorded when no males were engaged in courtship (courtship score of 0–1 on ethogram; see Table 1); minimum metabolic rate is the lowest metabolic rate recorded during periods in a courtship trial when at least four males were courting; average courtship metabolic rate is the average rate over the course of a courtship trial; maximum courtship metabolic rate is the instantaneous correction at the inflection point of the largest peak during courtship when at least four males were engaged in the highest level of courtship including caudocephalic waves and tail-searching behaviour prior to mating (see Table 1 for the ethogram). Box plots show 25% and 75% quartiles (boxes), medians (lines in the boxes), outermost values within the range of 1.5 times the respective quartiles (whiskers) and outliers (circles). Different letters above boxes denote significant differences.

Fig. 4 shows an example of GCS plotted through the duration of a trial (Fig. 4a) and an idealized GCS plot (Fig. 4b) based on average values from all 12 trials (Supplementary Table S1).

DISCUSSION

We tested the utility of whole-group measurements of metabolic rate for assessing energetic investment towards courtship and whether males adjust metabolic expenditure in response to competition. Male red-sided garter snakes show a significant increase in metabolic rate in the presence of competitors (SMR < RMR), and a further increase in the presence of a female (RMR < minimum courtship MR). Males seem to attain maximum metabolic rate in response to competition, but also periodically reduce courtship intensity in unison. This may be a way that males tailor their energetic expenditure relative to the likelihood of fitness payoffs while still maintaining their position in the mating ball.

Even at the lowest levels of courtship activity (i.e. minimum courtship metabolic rate), courtship MR was 1.8–3.6 times higher than baseline metabolic rates (0.1–4 J/min above RMR, 1–5.5 J/min above SMR). It is clear from our measure of factorial scope on maximum courtship effort that male red-sided garter snakes have a great capacity to upregulate their metabolism >17× in response to competition and female behaviour, using 10–58 J/min during the most frenetic courtship activity. Furthermore, our calculation of factorial increase suggests that males dramatically increase metabolic rate at the highest levels of courtship in response to

competition. We stress that our calculations of individual metabolic rates derived from group measurements are conservative and biased towards lower values. However, the highest metabolic rates were recorded when four to six males were all engaged in high-level courtship (all scored 4 on the ethogram, i.e. GCS = 16–24).

Our first-hand observations during the trials and subsequent detailed video analysis suggest that males oscillate between maximal courtship effort and periodic minimal courtship and rest phases. This may be to save energy, but also may be due to limited sustained aerobic capacity, which is likely to be challenging to maintain given the unusually high factorial increases we observed. In fact, some males seem to 'drop out' of competition entirely. For example, in Fig. 4a, it is evident that one male stopped courting for nearly 10 min after most of the group had engaged in vigorous courtship. This male then joined the group with maximum effort just prior to the female mating. This anecdote suggests that individuals have very different tactics that may reflect condition dependence and/or underlying innate physiological phenotypic differences. Indeed, males in better body condition are more likely to engage in vigorous courtship for longer and are thus more likely to mate (Shine et al., 2004).

Joy and Crews (1985) found that focal males increase their courtship intensity in response to the addition of more males to the aggregation; however, males did not increase their courtship intensity in unison with that of their competitors or with female movements (Joy & Crews, 1985). Nevertheless, we found that maximum MR and GCS often coincided with female movements. Indeed, MR was highest during intense courtship when males were
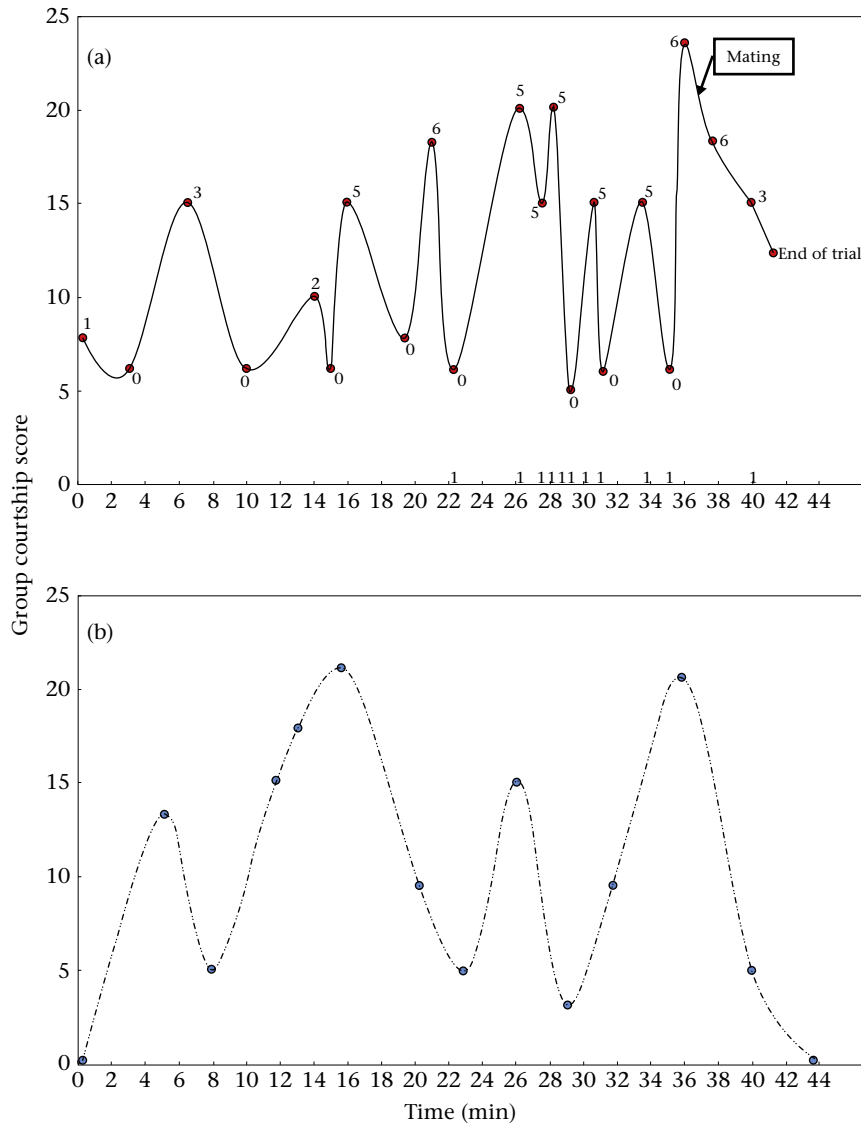
Figure 4. (a) Example of actual group ($N = 6$ males within a group) courtship score (GCS) data plotted on the Y axis through the duration of a trial. The points are time observations and are placed at the GCS. The number adjacent to each point is the number of males with a courtship score ≥ 3. The numbers above the X axis at the bottom of the graph indicate the numbers of males with a courtship score of 0. In this example, the same male stopped courting ~22 min into the trial and resumed courtship with a maximum courtship score at 36 min into the trial (all males had to court at level 4 to receive a GCS of 24). (b) Idealized GCS graph based on values averaged for all 12 trials (see Supplementary Table S1).

using tail-wrestling/searching behaviour, often in response to female movements. Matings only occurred when GCS was near the peak (>15) and average GCS was >15 during female movement, such as repositioning within the mating ball. Interestingly, tail-searching/wrestling behaviour is negatively correlated with mating success (Shine et al., 2004). It may seem counterintuitive that the highest metabolic rates corresponded with a behaviour that is negatively associated with mating success. However, tail-wrestling males may be displaced and lose cloacal apposition, which is the strongest determinate of mating success (Shine et al., 2004). Thus, males may increase muscular activity when the female is about to mate and/or when males have lost position and are striving to regain proper body alignment and cloacal apposition.

Minimum courtship MR was associated with what we have termed 'loafing' behaviour, in which males align with the female's body but remain relatively inactive, with only occasional short spasms of caudocephalic waves (every 60−90 s). The caudocephalic waves associated with 'loafing' are distinct in their low frequency and obvious lack of vigour compared with these same body waves

during vigorous courtship activity, which are much more forceful and can move the female's body. These waves have not been described before in the context of 'loafing' or when males are resting on a female. Presumably this is an adaptive tactic that allows a male to closely monitor the female's response to him while conserving the capacity to quickly respond to female movement or attempts by other males to dislodge him.

Male red-sided garter snakes are aphagous during the 2−4 weeks they remain at the emergence sites (O'Donnell, Ford, et al., 2004; O'Donnell, Shine, et al., 2004; Shine et al., 2001, 2006). To optimize their chances of mating, they need to maintain body condition, which is associated with the length of time they can remain at the emergence sites (Shine et al., 2004). Males are also judicious in which females they prefer to court; based on different sex pheromone profiles, males differentiate between, and are more likely to court, larger and unmated females (LeMaster & Mason, 2002; O'Donnell, Ford, et al., 2004; O'Donnell, Shine, et al., 2004; Shine, Phillips, et al., 2003). Such male discrimination likely optimizes energy expenditure relative to the potential fitness gains.

Large females are more fecund and can produce over 30 offspring per litter, whereas the smallest sexually mature females produce litters with as few as eight offspring (Friesen, Kerns, & Mason, 2014; Friesen, Mason, Arnold, & Estes, 2014). The females in this study were roughly the same size, so we did not test for the relationship between metabolic rate and female size, but we predict that future work aimed to test this hypothesis will find a positive relationship.

Empirical evidence demonstrates that male mate searching, courtship displays and territorial defence entail considerable energetic expense (Barske et al., 2014; e.g. Galimberti et al., 2007; Kotiaho et al., 1998; Lane, Boutin, Speakman, & Humphries, 2010; Marler, Walsberg, White, Moore, & Marler, 1995; Oberweger & Goller, 2001; Ryan, 1988; Shine & Mason, 2005; Vehrencamp, Bradbury, & Gibson, 1989). There is also growing evidence that polyandry escalates male energy expenditure on sperm production and ejaculates (Friesen et al., 2015; Hayward & Gillooly, 2011; Lüpold, 2013; Olsson, Madsen, & Shine, 1997; Wedell, Gage, & Parker, 2002). Our results suggest that courtship is a significant energetic cost for males, particularly given their aphagous condition and reliance on energy stores. Sustaining high metabolic rates depletes a male's finite energy stores, which limits the time he can spend at the emergence site and, in turn, reduces his mating opportunities within a season (Shine & Mason, 2005). Furthermore, there are implications for sex-specific life history trade-offs between reproduction and longevity as proposed by the disposable soma theory of ageing (Kirkwood & Austad, 2000; Kirkwood, 1977). Male red-sided garter snakes in this population may be sacrificing somatic maintenance for the possibility of gaining matings, a life history strategy differing from that of females and which may affect male longevity and lifetime reproductive success (Rollings et al., 2016). Using metabolic rates of female reproduction in garter snakes (Thamnophis sirtalis: Birchard, Black, Schuett, & Black, 1984; Van Dyke & Beaupre, 2011), Friesen et al. (2015) calculated that ejaculate production in this population requires more energy than maintaining a pregnancy, but they concluded that females probably have higher energetic expenditures for vitellogenesis. Based on our results from the current study, we suggest that total energy expenditure of male reproduction (ejaculates, behaviours and the development and maintenance of secondary sexual traits) may rival or exceed those of females, especially given the typically biennial reproduction by females of this species (Gregory, 2006).

Male energetic expenditure for reproduction may entail more physiological costs than are endured by females because of male fasting (e.g. less somatic maintenance and greater oxidative damage; Pascual, Pedrajas, Toribio, López-Barea, & Peinado, 2003; Rollings et al., 2016; Schull et al., 2016; Sharick et al., 2015; Sorensen et al., 2006). Male harassment behaviour directed at emerging females is understood as a hallmark sexual conflict (Shine, 2004; Shine, Langkilde, & Mason, 2003; Shine & Mason, 2005; Shine, O'Connor, & Mason, 2000). Future work should also quantify female energetic costs of courtship and test key predictions of facilitation of coerced matings by male caudocephalic wave-induced asphyxiation.

DECLARATION OF INTEREST

We, the authors, declare no conflicts of interest.

Supplementary material

Supplementary Material associated with this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2017.06.020.

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