

Energetic constraints and steroid hormone correlates of male calling behaviour in the túngara frog

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(With 4 figures in the text)

We investigated physiological constraints on calling behaviour in the túngara frog, *Physalaemus pustulosus*. First, we examined the hypothesis that energetic factors limit calling. Time spent in chorus activity is the best predictor of male mating success (Ryan, 1985). Manipulation of food availability demonstrated that males given supplemental food were more likely to call than males without supplemental food. Males without supplemental food were less likely to call even though their lipid reserves were only slightly less than males given the supplemental food, suggesting that part of the increased energy obtained from the supplemental food may have been channelled into calling to attract females. Secondly, we examined the role of steroid hormones in switching between reproductive and non-reproductive behaviours. Calling males had higher levels of plasma testosterone than silent males. Experimental elevation of corticosterone decreased testosterone levels and the likelihood of calling, thus linking both of these steroid hormones to calling behaviour. Males who responded to supplemental food by increasing calling had higher levels of testosterone than those without supplemental food, but there was no difference in plasma corticosterone levels. Overall, the results suggest that energetic factors affect calling behaviour and that one potential mechanism for this affect is through plasma testosterone levels. Corticosterone can decrease calling behaviour, however, corticosterone does not appear to mediate the effect of energetic factors on calling behaviour. These data are consistent with the hypothesis that energetic factors ultimately constrain sexual selection for calling behaviour.

Introduction

A principal area of research in the study of life history theory is analysis of trade-offs between traits that influence the individual fitness (Stearns, 1992), such as the potentially opposing selection pressures from sexual selection and natural selection (e.g. West Eberhard, 1979; Andersson, 1994). The role of energy allocation between these two conflicting pressures has been of particular interest. Because available energy must be partitioned between maintenance, growth, and reproduction, there may be limitations on the amount of energy a male can invest in attracting females, while still permitting sufficient energy for survival. Relatively few studies, however, have actually demonstrated whether energetic constraints can indeed limit sexually selected behaviours (e.g. review by Halliday, 1987; Ryan, 1988).

The túngara frog, *Physalaemus pustulosus*, has served as an especially useful model system for studying trade-offs between sexual selection (attractiveness to females) and survivorship (predation risk; Ryan, 1985). In *P. pustulosus*, as in many other frog species, the advertisement call

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functions both to attract females and repel males, thus calling behaviour is influenced by sexual selection (Rand, 1988; Ryan, 1991). Indeed, one of the primary factors limiting reproductive success in several species of frogs, including *P. pustulosus*, is the amount of time that males spend at a chorus (Greer & Wells, 1980; Kluge, 1981; Woodward, 1982; Arak, 1983; Godwin & Roble, 1983; Jacobson, 1985; Ryan, 1983, 1985; Gerhardt *et al.*, 1987). The breeding season of the túngara frog is seven months; however, males do not call continuously throughout it. During a 43-day period, males called an average of 7.2 days and spent an average of 35 days away from calling sites (Ryan, 1985). Males apparently do little if any feeding at the calling sites and there may be a time conflict between calling and foraging behaviour (Ryan, 1985), as occurs in other species of frogs (e.g. Woolbright & Stewart, 1987). One hypothesis for the cause of this variation is that calling is energetically costly and, after calling bouts, males need to replenish their energy stores (Bucher, Ryan & Bartholomew, 1982; Ryan, 1985), thus representing the need to balance sexual selection (mate attraction) and natural selection (maintaining energy stores necessary for survival). In support of this hypothesis, calling males expend considerably more energy than silent males (Bucher *et al.*, 1982), and calling has been found to be energetically costly in a number of other anurans (review by Pough *et al.*, 1992), such as in *Hyla microcephala* (Wells & Taigen, 1989). Trade-offs have also been found between calling and growth (e.g. Robertson, 1986; Given, 1988).

To date, however, it is unclear if energetic constraints actually limit calling time in male túngara frogs, and in one study, food supplementation had no effect on calling behaviour (Green, 1990). Moreover, potential physiological mechanisms (e.g. changes in circulating hormone levels) that might underlie energetically regulated variations in calling behaviour have yet to be explored.

Here we have used a variety of approaches to examine both environmental and physiological factors that may influence trade-offs between calling and non-calling behaviour, including available energy and plasma steroid levels. We manipulated the food supply to test if food availability could result in variation in calling behaviour, and we measured steroid hormone correlates of behaviour and of males exposed to different feeding regimes.

Methods

Food supplementation experiment

Enclosures were set up in Gamboa, Panama. Enclosures were made by covering 2 wading pools, approximately 1.8 m in diameter, with mosquito netting (to exclude predators). The enclosures were located on a slope approximately 2.5 m apart. To produce semi-natural conditions, half of each pool was covered with leaf litter and logs, and half with water so that males could move freely between the 2 areas. Food may have been available in the litter and logs. We captured 12 calling male *P. pustulosus* from nearby populations, and randomly divided the frogs into 2 groups (6/group), and placed each group into 1 of the 2 pools. One male disappeared before data collection. Males were individually marked using a method similar to that described by Ryan (1983), by suturing a small square of surveyor's tape with a unique colour code to the dorsal skin of the hypothermally anaesthetized frog.

We fed termites, the primary food source for túngara frogs (Ryan, 1985), to males in one of the pools 2–3 times per week by placing pieces of termite nests, approximately 9 cc, in the pools throughout the study. Males in this treatment are referred to as 'fed males'. Males appeared to consume the majority of termites soon after the termites were placed within the enclosure. We did not give supplemental food to males in pool 2; these are referred to as 'unfed males'. The treatments were reversed once between the 2 pools, using newly

captured frogs (9/group), to control for potential differences between pools. No remaining termites were observed in the enclosure for the unfed group in the second replicate. We present data for both replicates in the results.

We made behavioural observations from 18:00 to 24:00 h on days 12–16 (Replicate 1: 19–24 August 1990, Replicate 2: 30 August–3 September 1990) after starting the feeding regime using a method similar to that of Ryan (1983). Each hour during that time period we made a census to determine if individual males were calling (as indicated by being inflated) or not calling. That is, at the start of each hour, we scanned the enclosure until the location and behaviour of each individual was recorded. Thus, for each individual we obtained an estimate of the time spent calling: the number of censuses during which a male called divided by the total number of censuses (35). This approach, point-sampling (see Dunbar, 1976), allowed us to observe the behaviour of individuals within minutes of each other, and thus the effect of environmental factors known to influence calling behaviour other than the food manipulation, i.e. time of night or presence of predators (Tuttle, Taft & Ryan, 1982; Jennions & Backwell, 1992) were minimized.

We caught males at the end of the experiment and collected blood samples. Males were then killed using 3-aminobenzoic acid ethyl ester (MS222) and the carcasses frozen until lipid measurements were made.

Lipid analysis

We measured lipids in 3 groups: the 2 fed and unfed groups described in the food supplementation experiment and a third group caught in the wild at the end of the first replicate of the food supplementation experiment. Carcasses were dried in a Fisher Isotemp Oven set at 65 °C for 48 h, ground into small flakes with a mortar and pestle, and the neutral lipids extracted with petroleum ether by refluxing for 4 h with a Labconco Goldfish Fat Extraction Apparatus (e.g. Marler & Moore, 1989). Dried carcasses were weighed before and after lipid extraction and the differences used to estimate neutral lipid mass. Dry lean body mass was equal to the mass of the samples after removal of water and lipids.

Steroid hormone profiles

We collected blood samples from 18:00 to 23:00 h at the end of the feeding experiment to determine steroid hormone profiles of: a) calling versus silent males; and b) fed and unfed males. If a blood sample was not collected within 10 min after capture, no blood sample was collected for that male. We also recorded if males were calling or not calling the evening that the blood samples were collected. Blood samples were drawn by inserting a heparinized capillary tube into the orbital sinus. Blood samples were centrifuged within a few hours after collection and the resulting plasma (10–20 μ l) was decanted and stored at –20 °C. Radioimmunoassays were performed as described by Moore, M. C. (1986) following ether extraction of plasma and chromatographic separation of the steroid hormones from each other, and from interfering lipids, on diatomaceous earth:propanediol:ethylene glycol microcolumns. Intra-assay coefficients of variation ($N = 6$) were 7.0% for both testosterone and corticosterone. All samples were measured in one assay to reduce variability.

Corticosterone manipulation

We collected males on 12 September 1990 and randomly assigned them to receive 1 of 3 implants (Dow Corning Silastic medical grade tubing, 4 mm packed length, ID 1.47 mm, OD 1.96 mm): 1) 4 mm empty implant (control, $N = 9$); 2) 4 mm corticosterone implant (low corticosterone group, $N = 9$); 3) 4 mm corticosterone implant with a 0.5 mm wide hole punched into it (high corticosterone group, $N = 7$). Males were initially kept in the enclosures and later transported to the University of Texas at Austin. This move did not appear to cause any long-term changes in stress responses through corticosterone, as corticosterone levels were lower than those measured in frogs housed in the pool enclosures in Panama (see Figs 2 and 3). Males

were individually housed in adjacent $28 \times 15 \times 11$ cm containers which were tilted so that half of the container was filled with water. The other half contained crumpled paper towels and a glass container with fruit flies. We made hourly censuses on 26–28 September 1990 from 19:00–23:00 h to determine which males were calling and/or inflated. Males that were inflated were scored as calling (see Ryan, 1985). At the end of the study, we again recorded whether a male was calling or silent and collected blood samples (as previously described) from a subset of these males (Controls: $N = 7$, Low Corticosterone: $N = 5$, High Corticosterone: $N = 5$). Plasma hormone levels from all of the studies were measured after the corticosterone manipulation study.

Statistics

Data were analysed using the 'Crunch Interactive Statistical Package'. Behaviour and hormone levels were compared using non-parametric Mann–Whitney U-tests. One-tailed tests were used when a variable being measured was predicted to change in one direction only. A decrease in food availability would be predicted to result in a decrease in lipid stores, not an increase, therefore a one-tailed test was used for examining interactions between feeding and lipids. One-tailed tests were also used for comparing hormone levels between calling and silent males. Comparisons of body composition were made using *t*-tests. Data presented in figures are mean \pm S.E.

Results

Food supplementation experiment

We manipulated the amount of food available to calling males to assess if food availability limits calling behaviour. There were no significant differences in snout-vent lengths of males between the fed and unfed groups in either replicate (Replicate 1: $\bar{x} \pm$ S.E. of unfed males = 28.8 ± 0.5 mm, $\bar{x} \pm$ S.E. of fed males = 28.6 ± 0.5 mm, $U = 14.5$, $P = 93$, Replicate 2: $\bar{x} \pm$ S.E. unfed males = 28.6 ± 0.4 mm, $\bar{x} \pm$ S.E. fed males = 29.2 ± 0.4 mm, $U = 7.5$, $P = 0.32$). Within the fed group, 14 of the 15 males were observed consuming termites during the study. In the two replicates combined ($U = 36.0$, $P = 0.002$, fed group: 15 males, unfed group: 14 males) and in both Replicate 1 ($U = 4.0$, $P = 0.05$) and Replicate 2 ($U = 17.0$, $P = 0.04$), fed males were significantly more likely to call than unfed males (Fig. 1). The increase in food availability resulted in a four-fold increase in calling behaviour throughout the censusing period. During this time, four males in the unfed group and one male in the fed group did not call. In addition, males in the fed group called an average of 3.44 ± 0.37 days, whereas males in the unfed group called 1.77 ± 0.46 days ($U = 47.0$, $P = 0.01$). Thus the feeding regimes were correlated with both the overall amount of calling during the censusing period and with a decrease in the number of days on which males called. Non-calling males were found in the leaf litter and logs.

The increased food availability and calling behaviour was also associated with a significantly greater (1.3 times) neutral lipid mass and a non-significant trend (1.1 times) for more dry lean weight in fed males as compared to unfed males (Table I). There was no significant difference in lipid mass corrected for dry lean weight (Table I), possibly because of changes in both lipid and dry lean weight. As a comparison, males collected directly from the field at the end of the first replicate had a lipid mass that was $10.6 \pm 1.5\%$ ($\bar{x} \pm$ S.E.) of dry lean mass. There was no significant difference in lipid mass as a percentage of dry lean mass among the three groups (ANOVA, $F = 1.44$, $d.f. = 37$, $P = 0.24$). Individual estimates of calling behaviour were not correlated with lipid mass as a percentage of dry lean mass (Spearman Rank Correlation, $r = 0.19$, $d.f. = 27$, $P = 0.19$).

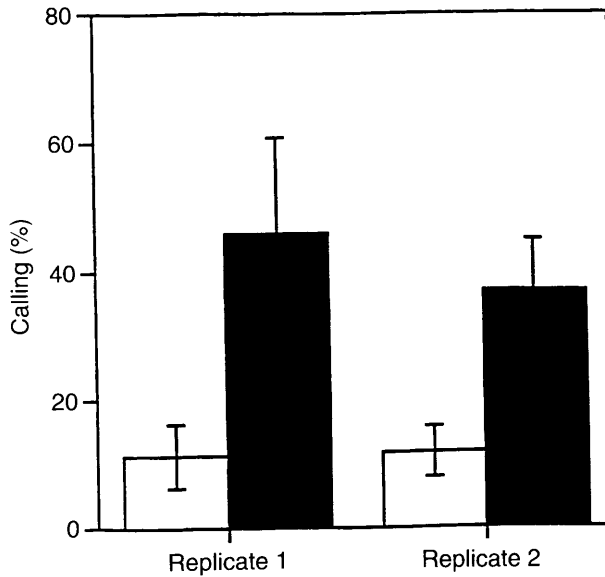


FIG. 1. Comparisons of calling behaviour between fed (■) and unfed (□) males. Calling (%) is the number of censuses in which a male was calling divided by the number of censuses taken. Fed males called significantly more in both replicates (see text).

Steroid hormone profiles

Comparisons between calling and silent males

To determine if differences in testosterone levels were associated with differences in calling behaviour, plasma testosterone was measured. Males that were calling on the day blood samples were collected had significantly higher levels of testosterone than silent males in both the food supplementation experiment (Fig. 2, one-tailed Mann-Whitney U-test $U = 40.5$, $P = 0.035$, calling males: $N = 9$; silent males: $N = 16$), and in the controls in the corticosterone manipulation experiment (Fig. 3, one-tailed Mann-Whitney U-test, $U = 0.0$, $P = 0.05$, calling males: $N = 5$; silent males: $N = 2$), as well as when all three groups in the laboratory experiment were combined (one-tailed Mann-Whitney U-test, $N = 9.0$, $P = 0.004$, calling males: $N = 9$; silent males: $N = 8$). In addition, there was a significant positive correlation between testosterone levels and individual estimates of calling behaviour throughout the censusing period (one-tailed Spearman Rank Correlation, $R = 0.42$, $d.f. = 25$, $P = 0.02$).

TABLE I

Comparisons of body composition between males with and without food supplementation using t -tests

	Not fed	Fed	t	$d.f.$	P
Lipid (g)	0.023 ± 0.00	0.030 ± 0.00	1.93	27	0.032
Dry lean mass	0.318 ± 0.02	0.355 ± 0.02	1.49	27	0.075
Lipid (% dry lean mass)	7.2 ± 1.0	8.8 ± 1.0	1.15	27	0.130

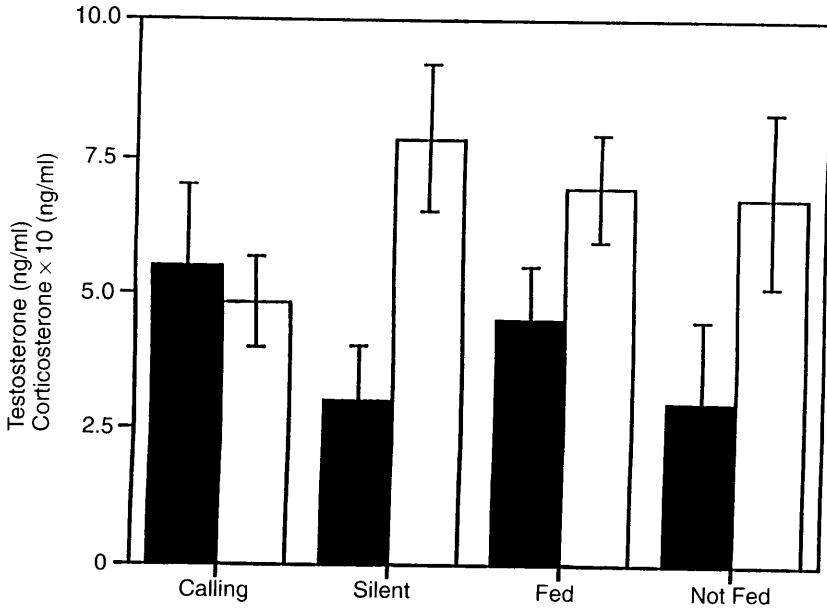


FIG. 2. Comparisons of testosterone (■) and corticosterone levels (□) in the food supplementation experiment. Levels were compared between calling and silent males (males calling or silent at the time of blood sampling) from all groups and replicates and between fed and unfed males.

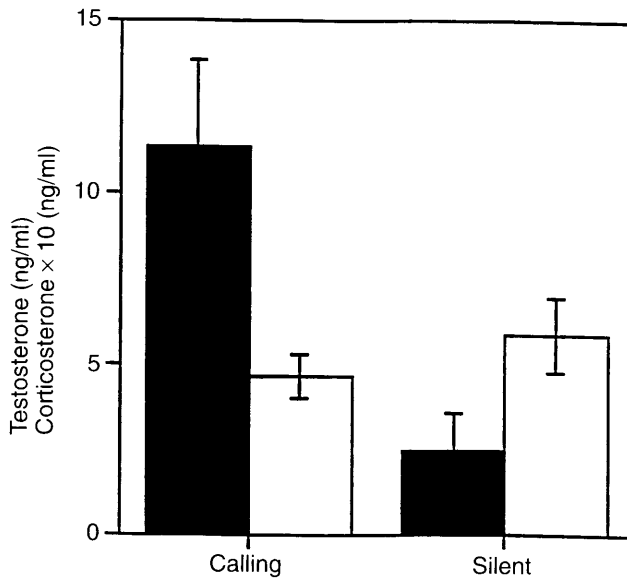


FIG. 3. Testosterone (■) and corticosterone (□) levels of calling and silent males in the control group (given empty implants) in the corticosterone implant study conducted in the laboratory. Calling and silent refers to the behaviour of the males at the time of blood sampling.

Plasma corticosterone levels in calling and silent males were measured to determine the relationship between corticosterone and calling behaviour (values are not compared in males given corticosterone implants). There was no significant difference in corticosterone levels between calling and silent males in the controls in the corticosterone manipulation experiment (Fig. 3, one-tailed Mann–Whitney U-test, $U = 2.0$, $P = 0.15$, calling males: $N = 5$; silent males: $N = 2$) and a non-significant trend in the food supplementation experiment (males from both fed and unfed groups, Fig. 2, $U = 48.0$, $P = 0.09$, calling males: $N = 9$; silent males: $N = 16$). Within the food supplementation experiment, there was no significant difference within the unfed group ($U = 8.0$, $P < 0.10$, calling males: $N = 2$; silent males: $N = 8$), but there was another non-significant trend in the fed group ($U = 14.0$, $P = 0.06$: calling males: $N = 7$; silent males: $N = 8$). In summary, in contrast to testosterone, there were no significant differences between plasma corticosterone levels of calling and silent males.

Comparisons between fed and unfed males

Males given supplemental food, a manipulation which promoted calling behaviour, had significantly higher levels of plasma testosterone than unfed males (Fig. 2, Mann–Whitney U-test, $U = 32.5$, $P = 0.02$, fed males: $N = 11$; unfed males: $N = 14$). In contrast, there was no significant difference in corticosterone levels between fed and unfed males (Fig. 2, $U = 72.5$, $P = 0.89$, fed males: $N = 11$; unfed males: $N = 14$), although levels were high in both groups, potentially masking differences between the groups. There was, however, no significant difference in the amount of time taken to bleed the males from the two groups (fed: $\bar{x} \pm \text{S.E.} = 6.7 \pm 1.1$ min, unfed: $\bar{x} \pm \text{S.E.} = 5.3 \pm 0.9$ min, $U = 58.0$, $P = 0.37$, fed males: $N = 11$; unfed males: $N = 14$) or in the corticosterone levels when corrected for bleeding time (comparisons of the residuals of the regression of time bled and corticosterone levels, fed: $\bar{x} \pm \text{S.E.} = -3.8 \pm 7.5$, unfed: $\bar{x} \pm \text{S.E.} = 5.7 \pm 13.6$, $U = 67.0$, $P = 0.68$). Interactions between corticosterone and testosterone were unclear because there was no significant correlation between corticosterone and testosterone plasma levels (Spearman Rank Correlation, $R = -0.29$, $d.f. = 25$, $P = 0.17$), although there was a significant negative correlation between corticosterone and testosterone in the corticosterone implant experiment (see below).

Corticosterone implant experiment

Although measurements of plasma corticosterone revealed no significant differences in corticosterone levels between calling and silent males, as described below, corticosterone implants had a significant impact on calling behaviour. Males given implants (combining both corticosterone groups) were significantly less likely to call than control males (Fig. 4a, $U = 26.5$, $P = 0.008$, controls: $N = 9$; low corticosterone group: $N = 9$; high corticosterone group: $N = 7$). There was, however, no significant difference in the estimated amount of calling between the two groups of males given implants (low and high corticosterone groups: $U = 22.0$, $P = 0.35$). Surprisingly, implants did not significantly increase measured levels of plasma corticosterone (Fig. 4b, one-tailed Kruskal Wallis, $H = 1.800$, $P = 0.20$, controls: $N = 9$; low corticosterone group: $N = 5$; high corticosterone group: $N = 5$), even though this experimental increase in corticosterone resulted in a significant decrease in testosterone levels (Fig. 4c, $H = 6.099$, $P = 0.02$), as well as the previously mentioned decrease in calling behaviour. High levels of corticosterone resulting from handling stress may, however, have masked differences in plasma corticosterone levels resulting from the implants.

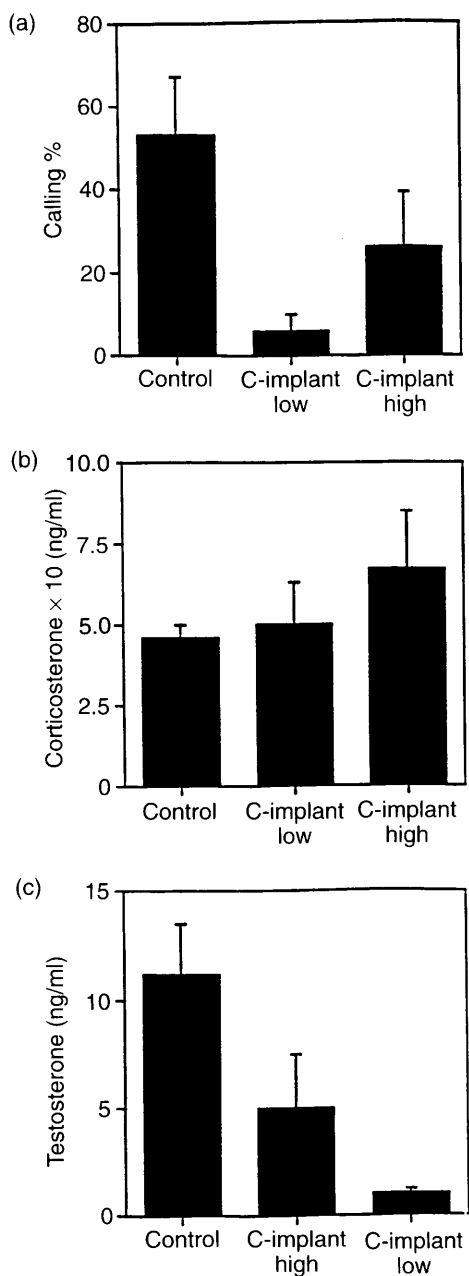


FIG. 4. (a) Calling %, (b) corticosterone levels, and (c) testosterone levels of males given empty implants (controls), males given 4 mm corticosterone implants (C-implant low), and males given 4 mm corticosterone implants with 0.5 mm holes punched into the implant (C-implant high). Calling (%) is the number of censuses in which a male was calling divided by the number of censuses taken.

Because corticosterone significantly reduced calling behaviour, and because calling behaviour was positively correlated with plasma testosterone, the effect of corticosterone implants on plasma testosterone was also examined. Implants resulted in a significant decrease in plasma testosterone levels (Fig. 4c). Thus males given implants typically called less and had reduced testosterone levels. There was a significant negative correlation between corticosterone and testosterone in the control group (Spearman Rank Correlation, $r = -0.81$, $d.f. = 5$, $P = 0.03$).

Discussion

The balance between sexually selected traits and energy limitations offer a potentially useful area for trade-off analysis. For example, it has been suggested, and in some cases directly demonstrated, that traits subject to sexual selection are energetically costly (see review by Halliday, 1987). There are, however, to date relatively few studies demonstrating that these costs cause variation in sexually selected traits (Halliday, 1987; Ryan, 1988). In this report, we have used calling in the túngara frog, *Physalaemus pustulosus*, to investigate potential interactions between sexual selection and energetic constraints. Sexual selection and communication in the túngara frog have been subjected to extensive study, and it has been hypothesized that calling behaviour (a sexually selected trait) is under energetic constraint (Ryan, 1985). Our results are consistent with this hypothesis. Simply put, males given supplemental food were significantly more likely to call than males not given supplemental food, even though there were only small differences in stored lipids (greater differences may have occurred in glycogen levels in the muscle). In addition, there was no correlation between lipid stores and calling behaviour, although this may be because absolute measurements of calling behaviour were not made. The lack of a large difference in stored lipid levels between fed and unfed males also suggests that the increased energy intake from the food supplements may have been channelled into calling behaviour. These results contrast with those of Green (1990), who did not document any effect of feeding on calling behaviour. There are three major differences between the two studies, which make comparisons difficult. First, the feeding regime used by Green was conducted over 3–5 days, as opposed to 12–16 days in this study. Secondly, Green placed males individually in jars positioned within a chorus each evening, whereas in this study, males were in larger enclosures more similar to the natural habitat with areas for both foraging and calling. Thirdly, Green monitored calling behaviour continuously in individuals using focal animal observations for four hours per night, as opposed to censusing the behaviour of males within a few minutes of each other for six hours per night. Our estimates of calling behaviour should, if anything, result in a bias towards finding no effect of energetics on calling behaviour. Any of these may have contributed to the differences in the results between the two studies. Green (1990) also released frogs used in the previously mentioned experiment and found no differences in return time to the chorus between males with different levels of food intake, perhaps because of variations in feeding behaviour after being released. Additional studies may be needed to investigate the causes for this variation in behavioural response to changes in food intake.

A rough estimate of the number of nights a male *P. pustulosus* can call based on his energy stores can be determined by estimating: 1) the amount of stored energy in the form of neutral lipids; and 2) the daily energy expenditure of males per day when calling. Calling males collected from their natural habitat had an average of 0.037 ± 0.005 g of lipid ($N = 10$). Therefore, on average, males had 1524.4 J of stored energy in the form of lipid, based on a conversion factor of

41.2 kJ/g lipid (Bradford, 1983; Pough *et al.*, 1992). If males expend 22.1 J/h while calling (Bucher *et al.*, 1982), call for six hours per night (Ryan, 1985), and expend 5.2 J/h during the rest of the day (Bucher *et al.*, 1982), then they would use approximately 226.2 J/day. Therefore, males caught in a chorus have sufficient stored energy from lipids to last for approximately 6.7 days of calling (glycogen stores such as those in the muscle probably also provide additional energy). Thus, because of the potential time conflict between calling and foraging (Ryan, 1985; Woolbright & Stewart, 1987), males may switch from calling to foraging behaviour before becoming energetically stressed because of severely decreased energy stores.

Our results suggest that variation in energy intake, such as ability to find food, can result in variations in calling behaviour. One potential scenario is that males call in a chorus, where available food is limited, until energy stores reach some minimal threshold (not solely lipid stores, as there was no correlation between lipid levels and individual levels of calling behaviour), and then leave the chorus to begin foraging (in our study significantly more of the unfed males were not calling and silent males were found among the leaf litter and logs). Calling males given supplemental food may be more likely to call because it takes longer to achieve the minimal energy threshold of lipid and glycogen stores. Because Green (1990) demonstrated that without any additional sources of food intake males can continue calling for at least five nights (four hours/night) of calling, they probably depend on a combination of lipids and glycogen to sustain calling behaviour. It is possible that glycogen stores are important over short-term periods, whereas lipid may become more important over longer periods of time (Pough *et al.*, 1992). It is also likely that the decision to call is influenced by other factors, such as predictability of food sources, or environmental and physiological variables such as predation (Tuttle & Ryan, 1981; Green, 1990; Jennions & Backwell, 1992) or disease. As mentioned previously, the amount of time spent in a chorus is a major determinant of a male's mating success. The primary implication of this study is that sexual selection on calling behaviour can be potentially balanced by the energetic demands that calling places on an animal over a time period longer than that examined in other studies.

In species in which individuals alternate reproductive and foraging behaviour over a time period of days, steroid hormones may be involved in balancing life-history trade-offs (Ketterson & Nolan, 1992). A combination of androgens and corticosterone could control this by stimulating and inhibiting behaviours. Alternatively, the absence of one hormone may simply permit other factors to have an effect. Testosterone levels are associated with sexual/aggressive behaviour in many species (reviews by Wingfield *et al.*, 1987; Sachs & Meisel, 1988), including poikilotherms (reviews by Moore & Deviche, 1988; Moore & Lindzey, 1992). In anurans, androgens are generally elevated during the breeding season, but fluctuations in plasma levels can occur such as in the toad, *Bufo japonicus*, in which androgens were relatively low when males left the breeding pond (Itoh & Ishii, 1990). In this study, we examined the pattern of testosterone in a frog species with an extended breeding season of seven months, during which males switch between calling at the breeding site and foraging for termites away from it (Ryan, 1985). Thus, the switch between behaviours may happen several times during the breeding season. We found that males calling in these semi-natural conditions have higher levels of testosterone than males found among the leaf litter and logs. This was also found to be true in males in the laboratory. Thus, even though androgens play a weaker role in controlling reproductive behaviour in amphibians (e.g. Wada & Gorbman, 1977; Moore, F. L., 1983, 1987; Wetzel & Kelley, 1983), as compared to other taxa, testosterone still appears to be correlated with relatively short-term changes (< 48 hours) in reproductive behaviour during the breeding season. Variation in

androgen levels may also be associated with variations in types of anuran reproductive behaviour such as territorial versus silent or satellite behaviour (Mendonca *et al.*, 1985), parental versus calling behaviour (Townsend & Moger, 1987), and amplexing versus non-amplexing males (Orchinik, Licht & Crews, 1988). We also measured steroid levels in males with different levels of available food and found that males with more available food had higher testosterone levels. These data are also consistent with the idea that high testosterone levels stimulate calling behaviour and lower levels may allow other factors to increase foraging behaviour. However, it is possible that increased food availability causes an increase in calling behaviour, which in turn stimulates testosterone production. Acoustic signals can at least influence androgen production in male grass frogs, *Rana temporaria* (Brzoska & Obert, 1980). Thus we have correlational evidence, but no causal evidence, supporting a role for testosterone in this behavioural switching.

What role does corticosterone play? Even though the implants did not result in significant differences in corticosterone levels, the effect of the corticosterone implants were clearly evident through the induced changes in calling behaviour, as well as the proportional decreases in testosterone with increased corticosterone administration. Corticosterone may have directly inhibited calling behaviour or its effect may have been through the decrease in testosterone levels. These results demonstrate that corticosterone can inhibit reproductive behaviour in anurans, as has been found in other taxa (reviews by Greenberg & Wingfield, 1987; Levine, Coe & Wiener, 1989; Lance, 1990), including rough-skinned newts (Moore & Miller, 1984). In addition, there was a non-significant trend ($P = 0.06$) for non-calling behaviour to be associated with high levels of corticosterone in fed males under semi-natural conditions, but not in unfed males. The results suggest that corticosterone can inhibit calling behaviour, but it is not clear at this stage whether corticosterone will only inhibit calling behaviour under certain conditions. More relevant to this study, however, is whether the energetic effects on behaviour are mediated through corticosterone. We did not find any correlational evidence indicating that corticosterone was associated with lower levels of available food in *P. pustulosus*. Thus, while testosterone may mediate the effects of energetics on behaviour, there is no evidence in this study for a role of corticosterone. Future studies will need to be done to establish hormonal control of switching between calling and foraging behaviour further, such as manipulating steroid levels and examining the effect on feeding behaviour.

In summary, we have provided evidence that switching between reproductive and non-reproductive behaviours can be influenced by levels of energy intake. We have correlational evidence that testosterone may be involved in this switching. Corticosterone also influences behavioural switching, however, there was no evidence that energetic stress influences behaviour through changes in corticosterone. We propose that energetic constraints act to counterbalance sexual selection for calling behaviour, perhaps acting through testosterone levels.

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