Sex differences in energetic costs explain sexual dimorphism in the circadian rhythm modulation of the electrocommunication signal of the gymnotiform fish *Brachyhypopomus pinnicaudatus*

Vielka L. Salazar* and Philip K. Stoddard

Department of Biological Sciences, Florida International University, University Park, Miami, FL 33199, USA

*Author for correspondence (e-mail: vsalaz01@fiu.edu)

SUMMARY

To understand the evolution of sexually dimorphic communication signals, we must quantify their costs, including their energetic costs, the regulation of these costs, and the difference between the costs for the sexes. Here, we provide the first direct measurements of the relative energy expended on electric signals and show for the focal species *Brachyhypopomus pinnicaudatus* that males spend a significantly greater proportion of their total energy budget on signal generation (11–22%) compared with females (3%). Both sexes significantly reduce the energy spent on electric signals during daylight hours through circadian modulation of the amplitude, duration and repetition rate of the electric signal, but this effect is more marked in males. Male body condition predicted the energy spent on electric signals (R^2=0.75). The oxygen consumed by males for signal production closely paralleled the product of the electric signal's waveform area (R^2=0.99) and the discharge rate (R^2=0.59), two signal parameters that can be assessed directly by conspecifics. Thus the electric communication signal of males carries the information to reveal their body condition to prospective mates and competing males. Because the electric signal constitutes a significant fraction of the energy budget, energy savings, along with predation avoidance, provides an adaptive basis for the production of circadian rhythms in electric signals.

Key words: gymnotiform, sexual dimorphism, circadian rhythm, electric fish, electric organ discharge, energetic cost, communication signal.

INTRODUCTION

Opposing selective forces shape the sexually dimorphic communication signals of many species. Mate choice and intrasexual competition often drive males' signals towards more conspicuous and costly forms whereas predation pressure and energetic cost generally drive signals of both sexes towards less conspicuous and less expensive forms (Andersson, 1994; Endler, 1978; Endler, 1980; Endler, 1983; Frischknecht, 1993; Houde, 1988; Kodric-Brown, 1993; Moodie, 1972; Pomiankowski, 1987; Prestwich, 1994; Ryan, 1988; Ryan et al., 1982; Searcy and Andersson, 1986). Electrocommunication signals, such as those displayed by weakly electric fish, likewise may have evolved in response to a similar suite of opposing selective pressures. Growing evidence suggests that hostile eavesdropping by predators has driven the evolution of electric signals (Hanika and Kramer, 1999; Hanika and Kramer, 2000; Reid, 1983; Stoddard, 1999) while exaggeration of male-typical electric signal traits may enhance reproductive opportunities (Curtis and Stoddard, 2003). Less well understood, however, is whether and how the energetic cost of electrogenesis has influenced the evolution and regulation of electric fish signals.

To date, no study has measured the energetic cost of electrogenesis relative to a fish's total energy budget and natural behaviors. Indeed, the few studies to address the cost of electric signals either have suggested that the costs are negligible (Hopkins, 1999; Julian et al., 2003) or have focused on estimating absolute costs of bioelectrogenesis with no reference to the overall energy budget (Aubert et al., 1961; Aubert and Keynes, 1968; Bell et al., 1976; Keynes, 1968). We undertook the first direct measurement of the energetic cost of electric signal production in weakly electric fish and examined this cost with respect to the overall energy budget and circadian rhythm in signal expression of this fish.

The nocturnal gymnotiform fish *Brachyhypopomus pinnicaudatus* (Hopkins, 1991) generates electric organ discharges (EODs) to electrolocate and communicate in the dark. During the breeding season, *B. pinnicaudatus* males display EODs of greater magnitude and duration than females (Franchina and Stoddard, 1998; Hopkins et al., 1990), and further exaggerate their EODs during the night-time hours of courtship and spawning (Franchina and Stoddard, 1998; Silva et al., 1999) (Fig. 1). In addition, various parameters of the EOD (amplitude, duration and repetition rate) oscillate with true circadian rhythms, the magnitudes of which are greater in males than in females (Silva et al., 2007; Stoddard et al., 2007). Therefore, heightened expression of circadian rhythmicity in the signals of males could have partially resolved the conflict between sexual selection for conspicuous signals and the attendant costs of predation and energetic expense. In a polygynous species, males should produce costly signals only at those times in the diel cycle when benefits are greatest (e.g. when females are receptive or males are competing), and females should strive to minimize signal costs at all times.

The enhanced EOD of males is associated with reproductive success. When given a choice between male *B. pinnicaudatus* of different sizes, females preferred the larger ones that also had greater EOD amplitudes and durations (Curtis and Stoddard, 2003). Furthermore, in males, body size correlates positively with the magnitude of the circadian rhythms in the EOD waveform's amplitude and duration (Franchina and Stoddard, 1998). Also, circadian rhythmicity of the EOD is affected by social interactions.
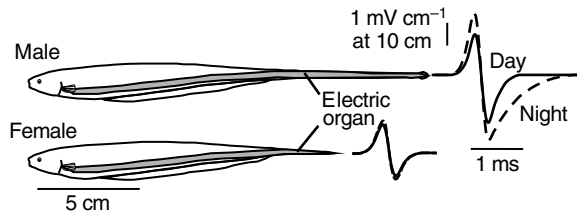
Fig. 1. The gymnotiform fish *Brachyhypopomus pinnicaudatus* shows strong sexual dimorphism in tail morphology and amplitude and duration of the electric organ discharge (EOD) waveform (Hopkins et al., 1990). Males show pronounced day–night differences in the EOD (Franchina and Stoddard, 1998). Shaded areas on each fish's body show the location of the bilateral electric organs.
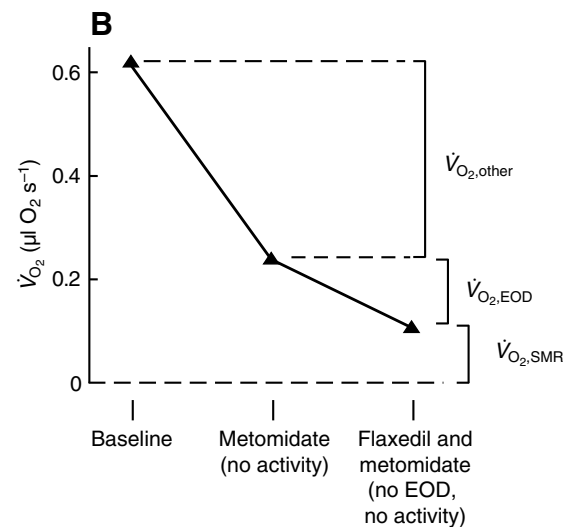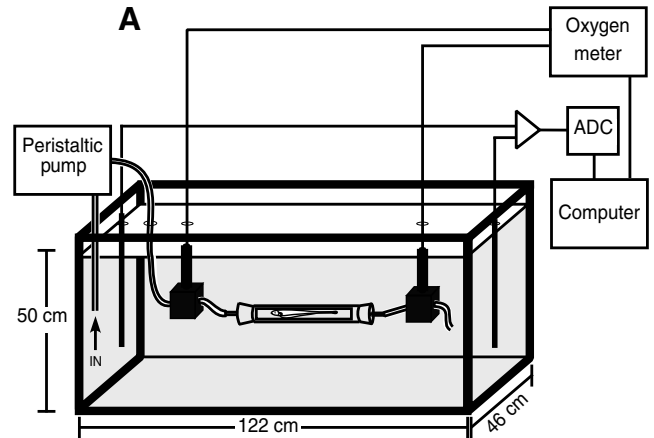

Fig. 2. (A) Schematic diagram of the respirometry tank set-up used to record EOD values and oxygen consumption rates simultaneously. EODs were digitized from carbon electrodes at opposite sides of the tank while the fish rested inside an unglazed ceramic tube. This tube was connected to a peristaltic pump and to the oxygen electrodes, which measured oxygen levels in the water as it was pumped at a constant flow rate. ADC, analog–digital converter. (B) Sample data from one male showing reduction of oxygen consumption with the two drug treatments. Metomidate abolishes activity, but not electrogenesis. Flaxedil, a curare analog, blocks the EOD as well. The difference between oxygen consumed at baseline with no drugs *versus* oxygen consumed following metomidate treatment is proportional to the energetic cost of ventilation and muscle tone plus any locomotion the fish attempts in the respirometry tube ($\dot{V}_{O_2,\text{other}}$). The difference between oxygen consumed following metomidate *versus* flaxedil and metomidate treatment ($\dot{V}_{O_2,\text{EOD}}$) is proportional to the energetic cost of electrogenesis at rest. The remaining oxygen consumption ($\dot{V}_{O_2,\text{SMR}}$) indicates the relative standard metabolic rate, the energetic cost of cellular metabolism at ambient temperature (27°C).

The enhanced EOD circadian rhythms observed in *B. pinnicaudatus* males attenuate gradually under social isolation and recover during social stimulation, and although social interactions with both sexes restore a socially isolated male's EOD circadian rhythm enhancement, male social stimuli induce a bigger and faster effect (Franchina et al., 2001). Furthermore, social interaction enhances the EOD discharge rate during the night (Silva et al., 2007). The EOD reduction observed during social isolation suggests that the nocturnal signal enhancements could be energetically costly, perhaps too costly to keep when no social benefit is to be gained (Terleph and Moller, 2003).

Therefore, we asked whether the daytime reductions in the electric signal waveform and repetition rate might save sufficient energy to confer selective advantage upon the male signaler and thus help to explain the existence of the well-documented sexually dimorphic circadian rhythms in the EOD waveforms and discharge rates of *Brachyhypopomus* (Franchina et al., 2001; Franchina and Stoddard, 1998; Hagedorn, 1995; Silva et al., 2007; Silva et al., 1999; Stoddard et al., 2003). To this end, we used respirometry and pharmacological manipulations to obtain direct measurements of the relative energy expended in electric signals.

MATERIALS AND METHODS
Experimental approach
We partitioned the energy budgets of 10 male and 10 female sexually mature *B. pinnicaudatus* by measuring EOD waveform, EOD repetition rate and the oxygen consumption rate ($\dot{V}_{O_2}$) while we applied successively two pharmacological agents that suppressed different energetic components: the $GABA_A$ binding enhancer (+)metomidate HCl suppressed locomotion, and the curare analog flaxedil blocked motor synapses on myogenic tissue including the electric organ, thus silencing the electric signal (details below). The body length of the tested males ranged from 19.0 to 27.0 cm and their body mass ranged from 11.8 to 17.3 g. Females' body length ranged from 13.9 to 18.4 cm and their body mass ranged from 6.6 to 13.6 g. Fish were fasted 24 h prior to the experiments, sufficient time for gut clearance. We measured and partitioned costs of activity, standard metabolic rate (SMR, minimum energy required to sustain basal physiological processes under a specified set of standard experimental conditions) and electrogenesis by day for both sexes.

EOD measurements
Fish were transported from the outdoor colony to an indoor aquarium (280 l, 122 cm×46 cm×50 cm length×width×height, filled with air-saturated water at a conductivity of 100 µS cm^{-1}) inside a temperature-controlled room (25°C) on a 12 h:12 h light:dark cycle. The EODs were detected by a pair of carbon rod electrodes (23 cm long, 6 mm diameter) located at opposite ends of the tank (Fig. 2A) and amplified by an AC-coupled differential amplifier (Charles Ward Electronics BMA-200, Ardmore, PA, USA), 1 Hz high-pass filter, 10 kHz low-pass filter, ×200 gain for males and ×500 gain for females. Online analysis was used to locate all the EODs in a 1 s train and calculate the mean values for the discharge rate, peak-to-peak amplitude (mV cm^{-1} at 10 cm) and

duration (ms). The duration of the EOD waveform was measured at 10% of the peak-to-peak amplitude to minimize sampling artifacts (Franchina et al., 2001; Franchina and Stoddard, 1998; Hopkins et al., 1990).

Power output (energy per EOD waveform × EOD repetition rate) of the electric organ (EOD power) could be calculated as the product of voltage and current. But EOD power cannot be measured directly or modeled simply in gymnotiform fish because the electric field is spatially complex (Stoddard et al., 1999). Fortunately, we can obtain repeatable measures of electric field strength distant from the fish (Franchina and Stoddard, 1998). From Ohm's Law, $I=V/r$, so current (I) varies directly with the electric field (V). Resistance (r) can be treated as a constant if water conductivity is constant (held at 100 μS cm^{-1}) and we assume tissue resistance is similar across individuals. With r constant, V^2 is proportional to power (VI), thus the time integral of V^2 is a valid and useful proxy of EOD power that lets us compare power output between individuals. This assumption is explored further in the Discussion. To obtain a representative proxy for electric field strength, we digitized head-minus-tail EOD waveforms of each fish across a 1.2 m length, using a calibrated and repeatable geometry (Franchina and Stoddard, 1998).

Daytime respirometry

We estimated the entire energy budget of active fish using oxygen consumption as a proxy for energy expenditure. To avoid adverse effects of social isolation on the fish's EOD (Franchina et al., 2001), a small male and a female were housed together with the test fish during the 24 h fasting period. An unglazed ceramic tube (3.2 cm i.d., 28.5 cm long, 223 ml) was used as the respirometry chamber because its interference with the fish's electric field is negligible. The respirometry apparatus consisted of a peristaltic pump (Masterflex L/S, Model 77200-62, Cole-Parmer Instrument Co., Vernon Hills, IL, USA), a pair of O$_2$-recording chambers with a temperature-compensated Clarke oxygen electrode (Analytical Sensors, Inc., model D012, Sugar Land, TX, USA) inside each one, and the respirometry chamber, all connected with Tygon® flexible plastic tubing (Fig. 2A). The gills of anesthetized fish (see below for procedure) were irrigated continuously with air-saturated water pumped into the mouth.

We measured the oxygen concentration in the water as the peristaltic pump drove air-saturated tank water through the entire apparatus at an average flow rate of 30 ml min^{-1}. A two-channel polarographic oxygen and temperature meter (Cameron Instruments Co., model OM200, Port Aransas, TX, USA) recorded readings from oxygen electrodes on the intake and outflow of the chamber (Fig. 2A). The oxygen electrode's zero end was calibrated once at the start of an experiment with a fresh solution of 100 mg of sodium sulfite per 5 ml of 0.1 mol l^{-1} sodium borate, while the high end (saturation level) was re-calibrated with air-saturated water. Data from the polarographic meter were simultaneously conveyed to the computer as it sampled and analyzed the electric signal data.

Pharmacological partition of the energy budget

After collecting control data from a resting fish ($\dot{V}_{O_2,total}$) using the respirometry apparatus, we induced the fish for 10 min with a 15 p.p.m. solution of (+)metomidate HCl, a GABA$_A$ binding enhancer that inhibits motor activity but not the EOD. Then the fish's gills were irrigated with a 5 p.p.m. maintenance solution of metomidate at a flow rate of 30 ml min^{-1} (Hattingh et al., 1975). We measured O$_2$ consumption rate ($\dot{V}_{O_2,metomidate}$) until it stabilized, typically 30 min after induction. At the concentration we used,

metomidate increases EOD amplitude by 10% over 2 h (M. R. Markham, personal communication), but our measurements were completed in 30 min, over which time metomidate has no measurable effect on the EOD. Next we completely silenced the EOD by injecting the fish intramuscularly with 3 μg g^{-1} fish of the curare analog flaxedil (gallamine triethiodide; Sigma, St Louis, MO, USA), a nicotinic acetylcholine receptor blocker. Again we recorded O$_2$ consumption ($\dot{V}_{O_2,metomidate\&flaxedil}$). At the end of a pharmacological experimental set, the test fish's gills were irrigated with air-saturated water until it recovered from the drug effects.

By subtracting oxygen consumption rates ($\dot{V}_{O_2}$) across the different pharmacological manipulations, we calculated the rates associated with the different energy budget components (Fig. 2B), standard metabolic rate ($\dot{V}_{O_2,SMR}=\dot{V}_{O_2,metomidate\&flaxedil}$), electric signal production ($\dot{V}_{O_2,EOD}=\dot{V}_{O_2,metomidate}-\dot{V}_{O_2,metomidate\&flaxedil}$), and everything else such as activity, muscle tone and ventilation ($\dot{V}_{O_2,other}=\dot{V}_{O_2,total}-\dot{V}_{O_2,SMR}-\dot{V}_{O_2,EOD}$). We used regression analysis to evaluate the relationship between EOD power and the dependent variable $\dot{V}_{O_2,EOD}$, during daytime. In addition, to assess the ancillary effects of the drugs we used, we reversed the order of drug treatment in five additional males.

Night-time methods

We measured oxygen consumption during normal nocturnal activity by sealing two fish of the same sex and size in a large tank. The fish were separated by a plastic screen to allow electrical interaction but to prevent fighting, which might suppress the loser's EOD. The tank was sealed with an acrylic lid that covered the entire water surface. One O$_2$ electrode was mounted in the lid so that oxygen concentration in the chamber could be recorded continuously for 9 h. The difference between the final and initial oxygen concentrations equaled the total oxygen consumed by the two fish. We assumed that fish in a pair consumed O$_2$ at a similar rate since they were matched by weight and length. Thus we divided the total O$_2$ of the fish pair by two to calculate the mean O$_2$ consumption rate for each fish during the night. We could not perform the daytime pharmacological manipulations at night because the night-time changes in EOD values are altered by light and handling, and diminish in the absence of social stimulation (Franchina et al., 2001). Instead, we used two alternative models to estimate the relationship between $\dot{V}_{O_2,EOD}$ and EOD power at night.

Models and data analyses

Model 1 assumes an individual's $\dot{V}_{O_2,EOD}$ increases linearly with the measured increase in EOD power at night. Model 2 assumes that the within-individual day-to-night changes follow the regression equations derived from the between-individual (same sex) data collected during the day (see Results, Fig. 3). For females, the two models produced indistinguishable values for modeled night-time $\dot{V}_{O_2,EOD}$, but for males, model 2 estimated mean night-time costs of electrogenesis to be about twice as high as those estimated by model 1. Both models assume that $\dot{V}_{O_2,EOD}$ varies linearly with discharge rate, an assumption we justify on theoretical and empirical grounds. EOD waveforms are not affected by discharge rate across the normal range of rates (Franchina and Stoddard, 1998) so the energetic cost of the EOD should be determined by the ATP required to actively transport the charge-carrying ions (Na$^+$ and K$^+$) back across excitable membranes following the action potentials. Empirically, $\dot{V}_{O_2,EOD}$ consumption varied linearly with EOD rate across males (Fig. 3C), though the data from females, with their low EOD power, were too noisy to establish a strong relationship (Fig. 3C). Even tighter relationships were obtained within individuals; however,
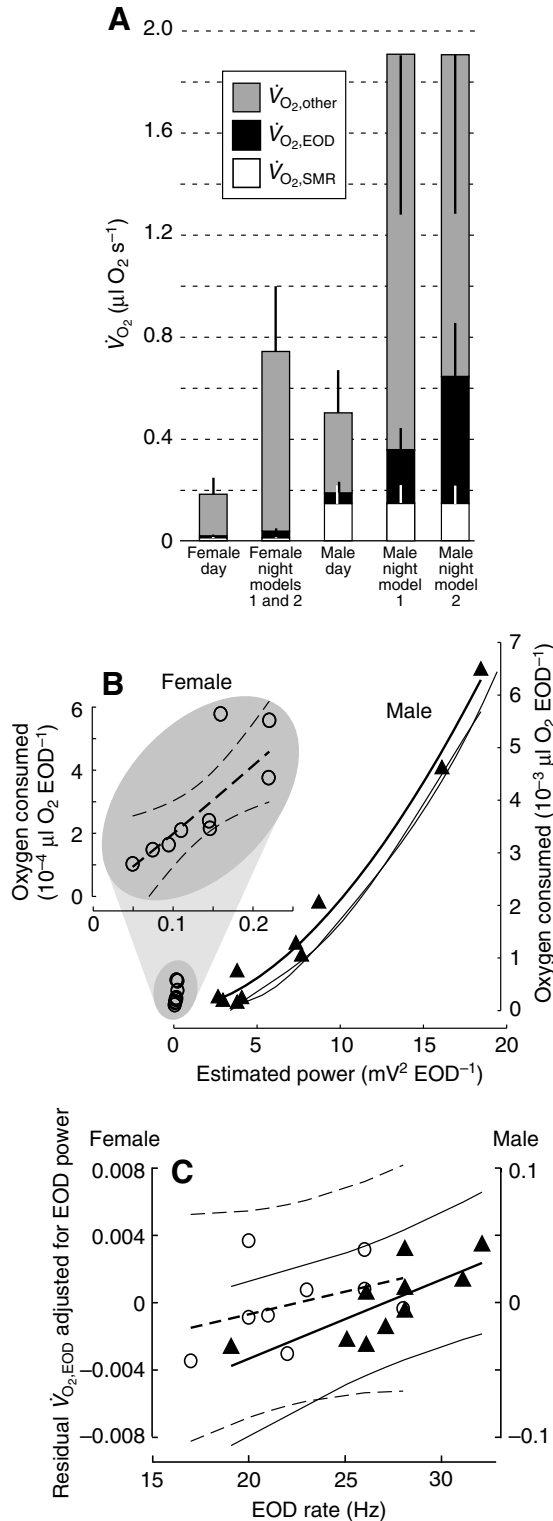
Fig. 3. (A) Measured and modeled means and 95% confidence intervals of sex differences in the day and night energetic costs of bioelectrogenesis ($\dot{V}_{O_2,EOD}$), standard metabolic rate ($\dot{V}_{O_2,SMR}$), and activity ($\dot{V}_{O_2,other}$). The energetic cost of electrogenesis is a small component of the total energy budget in females but a significant component in males. In both sexes, aerobic activity represented a big portion of the total budget and thus, as expected, $\dot{V}_{O_2,other}$ (which includes locomotion) increased dramatically at night. Energy budgets at night are modeled two ways: model 1 assumes electrogenesis costs increase linearly with EOD power; model 2 assumes individuals of each sex follow the same relationship between $\dot{V}_{O_2,EOD}$ and EOD power as that between individuals of the same sex (see B). (B) In both sexes the oxygen consumed per EOD, and thus its energetic cost, increases proportionally with the EOD power (males: y=3.32e–05$x^{1.80}$–1.03e–05, R^2=0.99; females: log$y$=1.06 log$x$–2.64, R^2=0.79). Comparing males and females with similar oxygen consumption per EOD indicates that males emit more powerful EODs. However, male costs increase exponentially as EOD power increases. Error lines represent the 95% confidence intervals. One female outlier (not shown) fell more than 4 s.d. beyond the regression line and was removed from all analyses. (C) In males, EOD rates are linearly related to residual $\dot{V}_{O_2,EOD}$ corrected for EOD power (R^2=0.56). The same relationship probably occurs in females as well, though the measured relationship does not significantly exceed measurement noise (R^2=0.15).

discharge rates (day 23.0 Hz for both sexes, night 75.6 Hz for males, 61.0 Hz for females, at 27°C).

All analyses and models were written in Matlab 7 with the Statistics Toolbox (script available upon request). Statistical analyses were all 2-tailed with α set to 0.05. All values are expressed as means and 95% confidence intervals. $\dot{V}_{O_2,EOD}$ values for females were normally distributed but those for males were not, thus we log transformed the male data to eliminate heteroscedasticity and used the transformed values in our analyses.

RESULTS
Sex differences in the energetic cost of electric signals
The EOD was more energetically expensive for males than for females, and more energetically costly at night than during the day. Total daytime $\dot{V}_{O_2}$ differed across the sexes (two-way ANOVA, dependent variable $\dot{V}_{O_2}$ and fixed factors sex and time of day: $F_{1,37}$=11.60, P=0.002) and total $\dot{V}_{O_2}$ differed significantly between day and night ($F_{1,37}$=20.71, P=0.0001). Although absolute costs of activity ($\dot{V}_{O_2,other}$) did not differ significantly between the two sexes (Wilcoxon rank-sum test, P=0.36), the cost of electrogenesis ($\dot{V}_{O_2,EOD}$) was much higher for males than for females (P=0.028), as was the standard metabolic rate ($\dot{V}_{O_2,SMR}$; P<0.0001; Fig. 3A).

Oxygen consumed per EOD ($\dot{V}_{O_2,EOD}$/rate in μl O_2 EOD^{-1}) varied tightly with measured EOD power (mV^2 cm^{-1}) in both sexes (Fig. 3B), confirming that the cost of electric signal production can be isolated accurately and measured with the methods we used. Oxygen consumed per EOD by males was best described by a power function of EOD power (y=3.32e–05$x^{1.80}$–1.03e–05, R^2=0.99, F>200, P<0.0001, where x is EOD power and y is oxygen consumed per EOD). For females, oxygen consumed per EOD was best described as a linear function of EOD power after log transformation (log$y$=1.06 log$x$–2.64, R^2=0.79, F=26.0, P=0.001). Controlling for EOD power, $\dot{V}_{O_2,EOD}$ varied linearly with EOD rate in males (R^2=0.56, F=10.02, P=0.013, Fig. 3C). This relationship was weak in females (R^2=0.15, F=1.26, P=0.3, Fig. 3C), perhaps because their values were closer to the noise floor of the measurement system.

The male EOD as a condition-dependent signal
Body condition is commonly estimated as mass adjusted for body length (Jakob et al., 1996; Kotiaho, 1999; Marshall et al., 1999).

these relationships are methodologically problematic because sufficiently variable EOD rates were only evident in untranquilized fish, in which locomotor activity is likely to covary with EOD rate. Because EOD rates were altered significantly by our handling and drug treatments, we rescaled the day and night values for $\dot{V}_{O_2,EOD}$ to comply with mean day and night rates recorded in our lab from resting and courting pairs of fish. We divided $\dot{V}_{O_2,EOD}$ values by obtained discharge rates then multiplied by the sex-typical mean
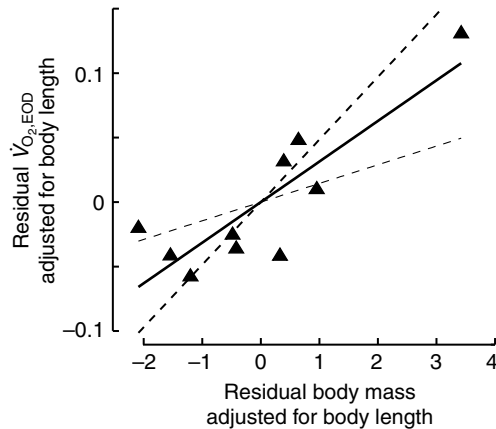
Fig. 4. Stepwise regression leverage plot shows residual male body mass and $\dot{V}_{O_2,EOD}$ after partialing out body length. Body mass adjusted for body length (i.e. how fat a male is for a given length) is a good indicator of body condition. How much energy a male is spending on electrogenesis can be inferred from the relationship between waveform and discharge rate (see Fig. 3B,C and Fig. 5B). Thus the amount of energy spent on electrogenesis, even during the day, can reveal a male's body condition. Dashed lines represent the 95% confidence interval around the regression line.
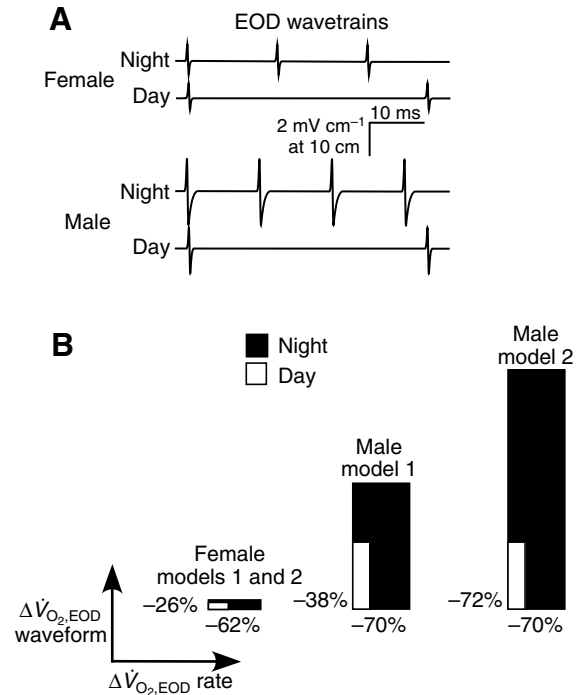

Fig. 5. (A) *B. pinnicaudatus* of both sexes decrease their discharge rates by day, and males in particular decrease the amplitude and duration of the individual waveforms. (B) Shown for each sex are the relative costs in energetic expense attributable to diurnal reductions of EOD discharge rate (abscissa), EOD waveform (ordinate), and the combination of the two (area).

While neither the male's body mass nor length alone was a good predictor of male $\dot{V}_{O_2,EOD}$ (mass: $R^2=0.34$, $F=4.16$, $P=0.08$; length: $R^2=0.05$, $F=0.43$, $P=0.53$), male body mass robustly predicted $\dot{V}_{O_2,EOD}$ after partialing out body length with stepwise linear regression ($R^2=0.75$, $F=10.29$, $P=0.008$, Fig. 4). The strong relationship between residuals of male body mass and O_2 consumed in electrogenesis suggests that the EOD is a condition-dependent signal, one for which males pay a premium incremental cost for increased signal power (exponent 1.8). Among females, by contrast, body mass was sufficiently correlated with body length that either factor alone predicted $\dot{V}_{O_2,EOD}$ (mass: $R^2=0.76$, $F=9.52$, $P=0.014$; length: $R^2=0.76$, $F=21.59$, $P=0.002$).

Sex differences in the energetic cost of different EOD components

Metabolic energy costs of the different EOD components differed strikingly between the sexes. During the night, the increase in the cost of electrogenesis in females resulted primarily from the night-time rise of their EOD repetition rates, whereas the cost increase in males resulted from the night-time rise in both their repetition rates and EOD waveforms (Fig. 5). Diurnal reduction of EOD rate lowered the total daytime cost of electrogenesis by 70% for males and about 62% for females (Fig. 5B). Diurnal reduction of EOD waveform amplitude and duration reduced the daytime cost of electrogenesis by another 38–72% for males (models 1 and 2) but only 26% for females (models 1 and 2 identical). The combined diurnal reduction in the cost of electrogenesis was 81–92% for males and 72% for females.

Based on model 1, the EOD as a mean percentage of the males' total energy budget increased from 8.3% by day (where daytime $\dot{V}_{O_2,EOD}$ was 0.0038, 0.0244 and 0.1814 μl O_2 s^{-1} minimum, median and maximum, respectively) to 12–26% at night (night-time $\dot{V}_{O_2,EOD}$ was 0.0131, 0.1095 and 0.3442 μl O_2 s^{-1} minimum, median and maximum, respectively). For females, the relative expense of the EOD remained unchanged between day and night, being 3.4% for both (daytime $\dot{V}_{O_2,EOD}$ was 0.0024, 0.0042 and 0.0145 μl O_2 s^{-1} minimum, median and maximum, respectively; and night-time

$\dot{V}_{O_2,EOD}$ was 0.0053, 0.0125 and 0.0642 μl O_2 s^{-1} minimum, median and maximum, respectively). Across 24 h, bioelectrogenesis consumed 11–22.5% of the males' total energy budget (where 24 h $\dot{V}_{O_2,EOD}$ in males was 0.0169, 0.1339 and 0.5256 μl O_2 s^{-1} minimum, median and maximum, respectively) but only 3.4% of the females' (24 h $\dot{V}_{O_2,EOD}$ in females was 0.0077, 0.0167 and 0.0787 μl O_2 s^{-1} minimum, median and maximum, respectively). Taken in the context of the entire energy budget, diurnal reduction of EOD pulse rate and waveform conferred a total daily energy saving of 7–16% for males and 2% for females (Fig. 3A and Fig. 5B).

DISCUSSION

How well do the drug treatments partition the energy budget?
The drugs we used to partition the energy budget target specific neurotransmitter systems rather than specific energetic compartments. Thus we do not expect perfect concordance between our methods and the true components of the energy budget. For instance, mean daytime $\dot{V}_{O_2,SMR}$ of males (0.138 μl O_2 s^{-1}) was over an order of magnitude higher than that of females (0.0085 μl O_2 s^{-1}). We were not surprised to find that males have higher SMR than females because of the high energetic demands of reproductive competition among males. But even after adjustment for differences in body mass, this difference is still larger than we expected from a sex difference in global cellular metabolism alone. The sex difference in SMR could also result from a substantially higher cost of maintaining the male's electric organ. Thus our calculated $\dot{V}_{O_2,SMR}$ values may include some maintenance components of the electric organ beyond costs per discharge and we may have underestimated both the overall cost and sex difference in the cost of electrogenesis. Other sources of error probably include the

differences in size, night-time activity levels, and signal strengths and rates between captive-reared and wild fish, as well as the proportionally higher measurement error for females due to their smaller EODs and lower metabolic rates. Curare analogs such as flaxedil probably quiet some cell groups in the brain and, as mentioned before, (+)metomidate HCl enhances the EOD waveform by 5–10% over 2 h. Any of these errors would change our numbers by a few per cent, but none of these is large enough to alter the general conclusions of our analysis.

Adaptive significance of the sex difference in the energetic cost of the different EOD components

Sex differences in the energetic costs of different EOD components may reflect the relative adaptive importance of each component in the life histories of the two sexes. In the sexually dimorphic species *B. pinnicaudatus*, EOD waveforms of females are very much like those of sexually undifferentiated juveniles. So, if one assumes that the female EOD has not been altered significantly by sexual selection, it follows that the energetic costs of the female EOD are primarily those needed for electrolocation. Costing only 3.5% of her total energy budget, the female's electric signal appears relatively inexpensive, as postulated previously for weakly electric fish in general (Bell et al., 1976; Julian et al., 2003). This logic also suggests that the female's nightly increase of EOD rate facilitates electrolocation, an assumption strongly supported by experimental data demonstrating enhanced sensory acuity with increased discharge rate (Caputi et al., 2003; Heiligenberg, 1980; Lissmann and Machin, 1958). Because mature male and female *B. pinnicaudatus* show similar electrolocation thresholds (Stoddard et al., 2006), we believe that sex differences in electric signals, and the higher energetic costs for males, are a sole consequence of sexual selection for communication.

The nightly increases in amplitude and duration of the EOD waveform may be adaptations to advertise the social and sexual status of males and to a lesser extent of females. In other gymnotiform species, both sexes display dominance hierarchies (Black-Cleworth, 1970; Hagedorn, 1986; Hagedorn and Heiligenberg, 1985; Hopkins and Westby, 1986; Westby, 1975). For instance, female *B. occidentalis* (a congener of *B. pinnicaudatus*) are known to be highly territorial (Hagedorn, 1988) and captive males compete for refuge sites (Hagedorn and Zelick, 1989). In *B. pinnicaudatus*, dominance interactions are common within both sexes, but we suspect they play a bigger role in the reproductive success of males, which appear to advertise their social status through their EOD waveforms. For instance, *B. pinnicaudatus* males increase their EOD waveform more when presented with another male than when presented with a female (Franchina et al., 2001) and the magnitude of the changes depends on the relative sizes of their respective waveforms at the onset of the interaction (V.L.S., unpublished data).

Sexual selection appears to have left multiple signatures on the electric signal production of males. Assuming the validity of our estimated $\dot{V}_{O_2,EOD}$ at night, EODs of males are not only more energetically expensive than those of females, but also make the males more conspicuous to electroreceptive predators by diverting significant signal energy into the spectral sensitivity range of electrosensory predators (Hanika and Kramer, 1999; Hanika and Kramer, 2000; Stoddard, 1999; Stoddard, 2002). It follows that the enhanced night-time EODs of males may provide honest indicators of male quality since those males that display nocturnal enhancement of the EOD incur the greatest energetic costs and predation risks. Another indicator of honest sexual signaling is that the energetic cost of the males' EODs is positively related to their body condition. Studies using experimental manipulations of the condition of males are needed to characterize the role of the nightly EOD enhancement in *B. pinnicaudatus* males as a condition-dependent signal.

Our use of the time integral of V^2 as a proxy of EOD power depends on the assumption that resistance (*r*) is constant across individuals. In fact, we believe that resistance is likely to be similar among members of each sex but to be systematically higher in females than males. Male *Brachyhypopomus pinnicaudatus* have longer and broader caudal filaments (tails) with flared tips, the 'feather-tails' for which they were named (Hopkins, 1991). These traits are thought to lower impedance between the electric organ and the surrounding water in male *B. pinnicaudatus* (Stoddard et al., 1999). Likewise, electrocytes of male *B. pinnicaudatus* have a lower membrane resistance than do those of females (M. R. Markham and P.K.S., unpublished data). Thus resistance is possibly an order of magnitude higher for females than for males. The effect of this difference can be seen in Fig. 3B as a rightward shift of female estimated EOD power towards the male values. For example, if $r_{female}=10r_{male}$, we would shift females 10 times to the right, causing females to overlap the low-end cluster of males in estimated EOD power. At the same time, because body plans and membrane resistances are similar among members of each sex, uncertainty about sex differences in resistance does not affect our figures or conclusions about the relationship between EOD waveform and energy consumption within each sex. Further, resistance has no bearing on our analysis of sex differences in energy allocation.

Adaptive significance of circadian rhythms in electric signal production

Male electric fish may enhance their EOD waveforms to advertise quality and may increase their EOD repetition rates both as signals of quality and to improve the acuity of electrolocation. But having improved the efficacy of a signal for its various functions, why reduce these parameters during the day? Both predation risk and metabolic energetic costs may account for the daytime signal reduction in males. Diurnal reductions in signal output (Franchina and Stoddard, 1998; Silva et al., 1999) confer significant energetic savings, especially in males as determined by our estimated night-time $\dot{V}_{O_2,EOD}$ values, a probable selective advantage of circadian rhythms in the different signal parameters. The males' EODs are not only more energetically expensive on average than those of females, but the low frequency spectral shifts, characteristic of the EOD waveform at night, render the signaler more conspicuous to electroreceptive predators (Hanika and Kramer, 1999; Hanika and Kramer, 2000; Stoddard, 1999), a common cost of sexual signals (Zuk and Kolluru, 1998). Nevertheless, if circadian rhythms in male EODs are solely adaptations to predation pressure, one might expect to find an inverse relationship between predation intensity and the sexually selected trait, in this case EOD circadian rhythms, similar to the signal trait variation seen in other fish such as guppies and sticklebacks (Candolin, 1997; Endler, 1978; Endler, 1980; Endler and Houde, 1995; Moodie, 1972). Males in populations subjected to strong predation pressure should display smaller EOD circadian rhythms on average (the EOD waveform should be less exaggerated at night to reduce conspicuousness, and the EOD repetition rate should be higher during the day to detect incoming predators), while those under low predation intensity should display more pronounced EOD circadian rhythms. Different geographical populations under different predation regimes often display rapid evolution, where predation pressure intensity leads to alternative life histories (Schoener et al., 2005; Yoshida et al., 2003). Perhaps such a pattern

may be present across *Brachyhypopomus* populations. Alternatively, if the magnitudes of circadian rhythms in the EODs remain unchanged across populations, regardless of differences in predation pressure, one would conclude that other selective pressures (i.e. energy conservation) sustain circadian oscillation of the sexually selected signal traits. Given that male *B. pinnicaudatus* from Uruguay and male *B. occidentalis* from Panama both display marked EOD circadian rhythms (Hagedorn, 1995; Silva et al., 1999), yet occupy habitats free of large electroreceptive predators (Eigenmann and Ward, 1905; Hagedorn, 1988; Silva et al., 2003), we suggest that the energetic cost of the enhanced night-time EOD is sufficient to sustain the persistence of strong circadian rhythms in the EODs of *Brachyhypopomus*.

How does the energetic cost of electric signals compare with the energetic cost of acoustic signals?

We assume that the EOD has been subjected to selective forces similarly documented in other communication models, such as insects, frog and birds, to mention a few (Andersson, 1994). We compared the energetic cost of electric signals to the well-characterized acoustic signals across different organisms to gain an understanding of where electric signals fall in reference to the energetic cost of communication signals in general. For the purposes of this comparison, we used 'signaling' factorial scope, the organism's $\dot{V}_{O_2,signal}$ divided by its $\dot{V}_{O_2,resting}$, where $\dot{V}_{O_2,signal}$ is the oxygen consumption when the animal is signaling and $\dot{V}_{O_2,resting}$ is the oxygen consumption when the animal is silent. During both conditions the animals are stationary. The signaling factorial scope is a good metric for comparative studies because it standardizes the energetic allocation across the effective metabolic demand of the organism by measuring the factor by which communication exceeds the metabolic cost at resting level. Analysis of the signaling factorial scopes across acoustic signalers compared with electric fish in this study indicates that the energetic cost of electric signals falls within the high end of the orthopteran range, within the lower end of the anuran range, approximately five times the energetic cost of crowing in a non-passerine bird, and twice the energetic cost of singing in a passerine bird (Table 1).

In contrast to acoustic signals, electric signals spread locally but do not propagate through the environment (Hopkins, 1986). The fish's electric organ creates an electrostatic field, the strength of which decreases by three orders of magnitude just 10 cm from the fish (Assad et al., 1999; Assad et al., 1998; Stoddard et al., 1999). An electric fish needs to increase its EOD field strength by a factor of eight to double the range of its electric field (Hopkins, 1986). Differences between the physical transmission properties of acoustic and electric signals may increase the relative cost of EODs over acoustic signals when they are used for sexual advertisement. Because electric signals enable electrolocation as well as communication, electric fish need to generate their EODs continuously to monitor changes in their surroundings. This dual function of electric signals increases selective pressure for low cost

Table 1. Comparison between the energetic cost of the electric signal of the gymnotiform *B. pinnicaudatus* and the Acoustic signals of various representative taxa

Species name	Common name	N	Mean mass (g)	Signal type	$\dot{V}_{O_2}$ signal (ml O_2 g^{-1} h^{-1})	$\dot{V}_{O_2}$ resting (ml O_2 g^{-1} h^{-1})	Factorial scope ($\dot{V}_{O_2,signal}/\dot{V}_{O_2,resting}$)	Ref.
Insecta – Orthoptera								
Anurogryllus arboreus	Trilling short-tailed cricket	12, 8	0.40	Acoustic call	3.576	0.315	11.35	8
Anurogryllus muticus	Trilling cricket	9	0.40	Acoustic call	9.06	1.7	5.33	7
Gryllotalpa australis	Trilling cricket	?	0.87	Acoustic call	4.883	0.42	11.63	6
Oecanthus celerinictus	Trilling tree cricket	5, 12	0.05	Acoustic call	2.931	0.437	6.71	8
Oecanthus quadripunctatus	Trilling tree cricket	9, 12	0.05	Acoustic call	3.342	0.476	7.02	8
Teleogryllus commodus	Chirping cricket	?		Acoustic call	1.17	0.85	1.38	7
Teleogryllus oceanicus	Chirping cricket	?		Acoustic call	0.27	1.25	0.22	7
Gryllus lineaticeps	Chirping variable field cricket	16	0.53	Acoustic call	0.774	0.464	1.67	4
Euconocephalus nasutus	Trilling katydid	12	0.68	Acoustic call	15.79	2.61	6.05	11
Neoconocephalus robustus	Trilling katydid	8	0.88	Acoustic call	13.88	1.92	7.23	11
Insecta – Lepidoptera								
Achroia grisella	Lesser wax moth	108	0.01	Ultrasonic call	2.32	1.34	1.73	10
Osteichthyes–Gymnotiformes								
Brachyhypopomus pinnicaudatus	Gymnotiform fish	10	14.1	EOD	0.074	0.014	5.29	This study
Amphibia – Anura								
Hyla cinerea	Green treefrog	10	5.1	Acoustic call	0.77	0.135	5.70	9
Hyla gratiosa	Barking treefrog	10	12.5	Acoustic call	1.12	0.105	10.67	9
Hyla squirella	Squirrel treefrog	9	2.6	Acoustic call	2.11	0.14	15.07	9
Hyla versicolor	Gray treefrog	13	8.62	Acoustic call	0.96	0.08	12.00	12
Hyperolius viridiflavus ommatostictus	Reed frog	18	1.4	Acoustic call	1.11	0.29	3.83	3
Physalaemus pustulosus	Túngara frog	10, 9	1.7	Acoustic call	0.35	0.31	1.13	1
Aves								
Thryothorus ludovicianus	Carolina wren	4	21	Song	9.3	3.56	2.61	2
Gallus gallus domesticus	Domestic rooster	13	1930	Crowing	0.15	1.02	0.15	5

$\dot{V}_{O_2,signal}$, signaling cost, i.e. metabolic rate during signalling; $\dot{V}_{O_2,resting}$, metabolic rate during resting; EOD, electric organ discharge; N, number of subjects tested. Whenever more than one N value is provided, the first N value corresponds to $\dot{V}_{O_2,signal}$ and the second to $\dot{V}_{O_2,resting}$. Signaling factorial scope ($\dot{V}_{O_2,signal}/\dot{V}_{O_2,resting}$) was calculated to determine how much metabolism had increased during calling when compared with rest. For poikilotherms, $\dot{V}_{O_2}$ data were obtained at a mean temperature of 25°C across all studies included here. References (Ref.): 1 (Bucher et al., 1982), 2 (Eberhardt, 1994), 3 (Grafe et al., 1992), 4 (Hoback and Wagner, 1997), 5 (Horn et al., 1995), 6 (Kavanagh, 1987), 7 (Lee and Loher, 1993), 8 (Prestwich and Walker, 1981), 9 (Prestwich et al., 1989), 10 (Reinhold et al., 1998), 11 (Stevens and Josephson, 1977), 12 (Taigen and Wells, 1985).

signals because turning off the electric signal for prolonged periods of time eliminates the active electric sense, increasing the risk of predation from all aquatic piscivores.

REFERENCES

Andersson, M. (1994). *Sexual Selection*. Princeton: Princeton University Press.

Assad, C., Rasnow, B., Stoddard, P. K. and Bower, J. M. (1998). The electric organ discharges of the gymnotiform fishes. II. Eigenmannia. *J. Comp. Physiol. A* **183**, 419-432.

Assad, C., Rasnow, B. and Stoddard, P. K. (1999). Electric organ discharges and electric images during electrolocation. *J. Exp. Biol.* **202**, 1185-1193.

Aubert, X. and Keynes, R. D. (1968). The temperature changes during and after the discharge of the electric organ in *Electrophorus electricus*. *Proc. R. Soc. Lond. B Biol. Sci.* **169**, 241-263.

Aubert, X., Fessard, A. and Keynes, R. D. (1961). The thermal events during and after the discharge of the electric organs of *Torpedo* and *Electrophorus*. In *Bioelectrogenesis* (ed. C. Chagas and A. Paes de Carvalho), pp. 136-146. Amsterdam: Elsevier.

Bell, C. C., Bradbury, J. and Russell, C. J. (1976). The electric organ of a mormyrid as a current and voltage source. *J. Comp. Physiol.* **110**, 65-88.

Black-Cleworth, P. (1970). The role of electric discharges in the non-reproductive behavior of *Gymnotus carapo*. *Anim. Behav. Monogr.* **3**, 1-77.

Bucher, T. L., Ryan, M. J. and Bartholomew, G. A. (1982). Oxygen consumption during resting, calling, and nest building in the frog *Physalaemus pustulosus*. *Physiol. Zool.* **55**, 10-22.

Candolin, U. (1997). Predation risk affects courtship and attractiveness of competing threespine stickleback males. *Behav. Ecol. Sociobiol.* **41**, 81-87.

Caputi, A. A., Aguilera, P. A. and Castelló, M. E. (2003). Probability and amplitude of novelty responses as a function of the change in contrast of the reafferent image in *G. carapo*. *J. Exp. Biol.* **206**, 999-1010.

Curtis, C. C. and Stoddard, P. K. (2003). Mate preference in female electric fish, *Brachyhypopomus pinnicaudatus*. *Anim. Behav.* **66**, 329-336.

Eberhardt, L. S. (1994). Oxygen consumption during singing by male Carolina wrens (*Thryothorus ludovicianus*). *Auk* **111**, 124-130.

Eigenmann, C. H. and Ward, D. P. (1905). The gymnotidae. *Proc. Wash. Acad. Sci.* **7**, 157-189.

Endler, J. A. (1978). A predator's view of animal color patterns. *Evol. Biol.* **11**, 319-364.

Endler, J. A. (1980). Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76-91.

Endler, J. A. (1983). Natural and sexual selection on color patterns in *Poecilia reticulata*. *Environ. Biol. Fishes* **9**, 173-190.

Endler, J. A. and Houde, A. E. (1995). Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**, 456-468.

Franchina, C. R. and Stoddard, P. K. (1998). Plasticity of the electric organ discharge waveform of the electric fish *Brachyhypopomus pinnicaudatus*. I. Quantification of day-night changes. *J. Comp. Physiol. A* **183**, 759-768.

Franchina, C. R., Salazar, V., Volmar, C. H. and Stoddard, P. K. (2001). Plasticity of the electric organ discharge waveform of the electric fish *Brachyhypopomus pinnicaudatus*: II. Social effects. *J. Comp. Physiol. A* **187**, 45-52.

Frischknecht, M. (1993). The breeding colouration of male three-spined sticklebacks (*Gasterosteus aculeatus*) as an indicator of energy investment in vigour. *Evol. Ecol.* **7**, 439-450.

Grafe, T. U., Schmuck, R. and Linsenmair, K. E. (1992). Reproductive energetics of the African reed frogs, *Hyperolius viridiflavus* and *Hyperolius marmoratus*. *Physiol. Zool.* **65**, 153-171.

Hagedorn, M. (1986). The ecology, courtship and mating of gymnotiform electric fish. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 497-525. New York: Wiley.

Hagedorn, M. (1988). Ecology and behavior of a pulse-type electric fish, *Hypopomus occidentalis* (Gymnotiformes, Hypopomidae), in a freshwater stream in Panama. *Copeia* **2**, 324-335.

Hagedorn, M. (1995). The electric fish *Hypopomus occidentalis* can rapidly modulate the amplitude and duration of its electric organ discharges. *Anim. Behav.* **49**, 1409-1413.

Hagedorn, M. and Heiligenberg, W. (1985). Court and spark: electric signals in the courtship and mating of gymnotoid fish. *Anim. Behav.* **33**, 254-265.

Hagedorn, M. and Zelick, R. (1989). Relative dominance among males is expressed in the electric organ discharge characteristics of a weakly electric fish. *Anim. Behav.* **38**, 520-525.

Hanika, S. and Kramer, B. (1999). Electric organ discharges of mormyrid fish as a possible cue for predatory catfish. *Naturwissenschaften* **86**, 286-288.

Hanika, S. and Kramer, B. (2000). Electrosensory prey detection in the African sharptooth catfish, *Clarias gariepinus* (Clariidae), of a weakly electric mormyrid fish, the bulldog (*Marcusenius macrolepidotus*). *Behav. Ecol. Sociobiol.* **48**, 218-228.

Hattingh, J., Fourie, F. L. and Van Vuren, J. H. (1975). The transport of freshwater fish. *J. Fish Biol.* **7**, 447-449.

Heiligenberg, W. (1980). The evaluation of electroreceptive feedback in a gymnotoid fish with pulse-type electric organ discharges. *J. Comp. Physiol. A* **138**, 173-185.

Hoback, W. W. and Wagner, W. E. J. (1997). The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. *Physiol. Entomol.* **22**, 286-290.

Hopkins, C. D. (1986). Temporal structure of non-propagated electric communication signals. *Brain Behav. Evol.* **28**, 43-59.

Hopkins, C. D. (1991). *Hypopomus pinnicaudatus* (Hypopomidae) a new species of gymnotiform fish from South America. *Copeia* **1**, 151-161.

Hopkins, C. D. (1999). Design features for electric communication. *J. Exp. Biol.* **202**, 1217-1228.

Hopkins, C. D. and Westby, G. W. M. (1986). Time domain processing of electric organ discharge waveforms by pulse-type electric fish. *Brain Behav. Evol.* **29**, 77-104.

Hopkins, C. D., Comfort, N. C., Bastian, J. and Bass, A. H. (1990). Functional analysis of sexual dimorphism in an electric fish, *Hypopomus pinnicaudatus*, order Gymnotiformes. *Brain Behav. Evol.* **35**, 350-367.

Horn, A. G., Leonard, M. L. and Weary, D. M. (1995). Oxygen consumption during crowing by roosters: talk is cheap. *Anim. Behav.* **50**, 1171-1175.

Houde, A. E. (1988). The effects of female choice and male-male competition on the mating success of male guppies. *Anim. Behav.* **36**, 888-896.

Jakob, E. M., Marshall, S. D. and Uetz, G. W. (1996). Estimating fitness: a comparison of body condition indices. *Oikos* **77**, 61-67.

Julian, D., Crampton, W. G. R., Wohlgemuth, S. E. and Albert, J. S. (2003). Oxygen consumption in weakly electric Neotropical fishes. *Oecologia* **442**, 502-511.

Kavanagh, M. W. (1987). The efficiency of sound production in two cricket species, *Gryllotalpa australis* and *Teleogryllus commodus* (Orthoptera: Grylloidea). *J. Exp. Biol.* **130**, 107-119.

Keynes, R. D. (1968). The temperature changes during and after the discharge of the electric organ in *Malapterurus electricus*. *Proc. R. Soc. Lond. B Biol. Sci.* **169**, 265-274.

Kodric-Brown, A. (1993). Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behav. Ecol. Sociobiol.* **32**, 415-420.

Kotiaho, J. S. (1999). Estimating fitness: comparison of body condition indices revisited. *Oikos* **87**, 399-400.

Lee, H. J. and Loher, W. (1993). The mating strategy of the male short-tailed cricket *Anurogryllus muticus* De Geer. *Ethology* **95**, 327-344.

Lissmann, H. W. and Machin, K. E. (1958). The mechanism of object location in *Gymnarchus niloticus* and similar fish. *J. Exp. Biol.* **35**, 451-486.

Marshall, S. D., Barrow, J. H., Jakob, E. M. and Uetz, G. W. (1999). Re-estimating fitness: can scaling issues confound condition indices? *Oikos* **87**, 401-402.

Moodie, G. E. E. (1972). Predation, natural selection and adaptation in an unusual threespine stickleback. *Heredity* **28**, 155-167.

Pomiankowski, A. (1987). The cost of choice in sexual selection. *J. Theor. Biol.* **128**, 195-218.

Prestwich, K. N. (1994). The energetics of acoustic signaling in anurans and insects. *Am. Zool.* **34**, 625-643.

Prestwich, K. N. and Walker, T. J. (1981). Energetics of singing in crickets: effects of temperature in three trilling species (Orthoptera: Gryllidae). *J. Comp. Physiol. B* **143**, 199-212.

Prestwich, K. N., Brugger, K. E. and Topping, M. (1989). Energy and communication in three species of hylid frogs: power input, power output and efficiency. *J. Exp. Biol.* **144**, 53-80.

Reid, S. (1983). La biologia de los bagres rayados *Pseudoplatystoma fasciatum* y *P. tigrinum* en la cuenca del rio Apure-Venezuela. *Rev. Unellez Cienc. Tecnol.* **1**, 13-41.

Reinhold, K., Greenfield, M. D., Jang, Y. and Broce, A. (1998). Energetic cost of sexual attractiveness: ultrasonic advertisement in wax moths. *Anim. Behav.* **55**, 905-913.

Ryan, M. J. (1988). Energy, calling and selection. *Am. Zool.* **28**, 885-898.

Ryan, M. J., Tuttle, M. D. and Rand, A. S. (1982). Bat predation and sexual advertisement in a neotropical anuran. *Am. Nat.* **119**, 136-139.

Schoener, T. W., Losos, J. B. and Spiller, D. B. (2005). Island biogeography of populations: an introduced species transforms survival patterns. *Science* **310**, 1807-1809.

Searcy, W. A. and Andersson, M. (1986). Sexual selection and the evolution of song. *Annu. Rev. Ecol. Syst.* **17**, 507-533.

Silva, A., Quintana, L., Galeano, M., Errandonea, P. and Macadar, O. (1999). Water temperature sensitivity of the EOD waveform in *Brachyhypopomus pinnicaudatus*. *J. Comp. Physiol. A* **185**, 187-198.

Silva, A., Quintana, L., Galeano, M. and Errandonea, P. (2003). Biogeography and breeding in Gymnotiformes from Uruguay. *Environ. Biol. Fishes* **66**, 329-338.

Silva, A., Perrone, R. and Macadar, O. (2007). Environmental, seasonal, and social modulations of basal activity in a weakly electric fish. *Physiol. Behav.* **90**, 525-536.

Stevens, E. D. and Josephson, R. K. (1977). Metabolic rate and body temperature in singing katydids. *Physiol. Zool.* **50**, 31-42.

Stoddard, P. K. (1999). Predation enhances complexity in the evolution of electric fish signals. *Nature* **400**, 254-256.

Stoddard, P. K. (2002). Electric signals: predation, sex, and environmental constraints. In *Advances in the Study of Behaviour*. Vol. 31 (ed. P. J. B. Slater, J. S. Rosenblatt, C. T. Snowdon and T. J. Roper), pp. 201-242. New York, London: Academic Press.

Stoddard, P. K., Rasnow, B. and Assad, C. (1999). Electric organ discharges of gymnotiform fishes: III. *Brachyhypopomus*. *J. Comp. Physiol. A* **184**, 609-630.

Stoddard, P. K., Markham, M. R. and Salazar, V. L. (2003). Serotonin modulates the electric waveform of the gymnotiform electric fish *Brachyhypopomus pinnicaudatus*. *J. Exp. Biol.* **206**, 1353-1362.

Stoddard, P. K., Zakon, H. H., Markham, M. R. and McAnelly, L. (2006). Regulation and modulation of electric waveforms in gymnotiform electric fish. *J. Comp. Physiol. A* **192**, 513-624.

Stoddard, P. K., Markham, M. R., Salazar, V. L. and Allee, S. (2007). Circadian rhythms in electric waveform structure and rate in the electric fish *Brachyhypopomus pinnicaudatus*. *Physiol. Behav.* **90**, 11-20.

Taigen, T. L. and Wells, K. D. (1985). Energetics of vocalization by an anuran amphibian (Hyla versicolor). *J. Comp. Physiol. B* **155**, 163-170.

Terleph, T. A. and Moller, P. (2003). Effects of social interaction on the electric organ discharge in a mormyrid fish, *Gnathonemus petersii* (mormyridae, Teleostei). *J. Exp. Biol.* **206**, 2355-2362.

Westby, G. W. M. (1975). Comparative studies of the aggressive behaviour of two gymnotoid electric fish (*Gymnotus carapo* and *Hypopomus artedi*). *Anim. Behav.* **23**, 192-213.

Yoshida, T., Jones, L. E., Ellner, S. P., Fussmann, G. F. and Hairston, N. G., Jr (2003). Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* **424**, 303-306.

Zuk, M. and Kolluru, G. R. (1998). Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* **73**, 415-438.