

# Do female frogs have higher resting metabolic rates than males? A case study with *Xenopus allofraseri*

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## Keywords

anurans; metabolic rate; respirometry; RMR; sexual dimorphism; *Xenopus allofraseri*.

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## Abstract

The energetic costs of body maintenance can have a profound influence on the energy that an individual can allocate to other functions such as growth, locomotion, or reproduction. Therefore, resting metabolism can ultimately affect an individual's survival or reproductive success, especially when food is limited. Although males and females often differ in their body composition (e.g. sex organs and fat accumulation) and body size, the occurrence and direction of sexual dimorphism in resting metabolism remains poorly understood in anurans. In the present study, we investigated whether males and females of the false Fraser's clawed frog *Xenopus allofraseri* differ in their resting metabolic rates. We used an open-flow push-through respirometry system to measure the volume of carbon dioxide (VCO<sub>2</sub>) produced by animals at rest. Variation in VCO<sub>2, min</sub> was explained by ambient temperature, body size, and sex. For a similar body size, our results revealed that females had about a 50% higher resting metabolic rate (RMR) than males. We suggest that the enhanced investment in gamete production in females compared to males may explain this difference. We further suggest that a lower RMR in males compared to females at similar body size could be selectively advantageous as unallocated energy may be devoted to costly mating, in support of the 'compensation' hypothesis.

## Introduction

Resting metabolic rate (RMR) corresponds to the minimal energy required for body maintenance. This self-maintenance cost may in turn restrict energy allocation to other essential processes such as growth, locomotion, or reproduction (Zaidan, 2003; Finkler, 2006; Beaupre & Zaidan, 2012; Louppe *et al.*, 2018), which ultimately may affect an individual's behavior (Careau *et al.*, 2008; Reale *et al.*, 2010; Mathot *et al.*, 2015) or reproductive success (Ophir *et al.*, 2010; Rønning *et al.*, 2016). In this context, three principal variables are often investigated, or controlled, for their influence on RMRs: temperature, body mass, and feeding. However, some variables can be confounded with one another. For example, in house sparrows (*Passer domesticus*) individuals with higher metabolic rates have reduced number of offspring, but in a sex-dependent manner (Rønning *et al.*, 2016). This relationship in females still holds after correcting for body mass differences between sexes. However, it often remains challenging to tease apart the effect of sex from those of body mass as in dimorphic species one of the sexes is typically heavier than the other.

Differences in RMR between the sexes have been broadly studied among vertebrates including birds (Rønning *et al.*,

2016), reptiles (Garland & Else, 1987; Hare *et al.*, 2010), mammals (Sadowska *et al.*, 2005; Boratynski & Koteja, 2010; Jagim *et al.*, 2018), and invertebrates (Canals *et al.*, 2015; Tomlinson & Menz, 2015). Interestingly, the direction of differences between males and females varies among species, raising questions on the proximate factors driving the dimorphism in resting metabolism. Regarding amphibians, most studies have focused on salamanders (Finkler *et al.*, 2003) and showed either higher metabolism in gravid females (Finkler *et al.*, 2002; Finkler, 2006) or post-gravid females (Finkler *et al.*, 2003) than in males or no sex differences (Ryan & Hopkins, 2000). However, studies of sexual dimorphism in RMR in anurans are scant (but see Grafe *et al.*, 1992; Finkler *et al.*, 2014). Yet, it is known that investment in reproduction is generally more substantial for females than males, primarily because of the higher mass of eggs compared to sperm. For instance, reed frog (*Hyperolius* spp.) females invest almost 30% of assimilated energy into clutches (Grafe *et al.*, 1992). Gametogenesis in both sexes is driven by gonadotropins, but is more dependent upon net energy balance in females than males, at least in temperate zone anurans (Jørgensen *et al.*, 1979). One study exploring RMR in cane toads during the breeding season showed that RMR was not different between

sexes, yet the positive relationship between (dry) body mass and oxygen consumption at rest was higher in males than females suggesting differences in the ability to allocate resources to physical activity, mate finding, or gametogenesis (Finkler *et al.*, 2014). However, outside the breeding season, in a non-reproductive state and under resting condition, it remains unclear whether male and female anurans show differences in resting metabolic rate.

In the present study, we investigated whether males and females of the false Fraser's clawed frog *Xenopus allofraseri* differ in their resting metabolic rates under controlled laboratory conditions. We predict females to have higher RMR than males for a given size due to the higher cost of gametogenesis in females.

## Materials and methods

### Field collection and laboratory maintenance

Males and females of *X. allofraseri* were caught in Cameroun in December of 2009 and brought back to the Function and Evolution (FUNEVOL) laboratory at the Muséum National d'Histoire Naturelle, Paris, France. In previous studies, we referred to these animals as *Xenopus tropicalis* based on the collection locality and external morphological traits, yet recent transcriptomic data showed unambiguously that these animals are *X. allofraseri*. Animals were maintained in groups of 6 to 10 individuals of similar sex, in 50-liter aquaria in *Xenopus* standalone racks with a recirculation system (Aquanearing) and under controlled ambient temperature (24°C, similar to the temperature of water bodies measured in the field). The frogs were given one month to recover and were then pit-tagged (Nonatec, Rodange, Luxembourg) allowing the unique identification of each individual. Frogs were fed twice weekly with beef heart, earthworms, or mosquito larvae, and we ensured they were fasted 3 days prior to the experiment. All animals were sexually mature at the time of experimentation in spring of 2017, yet their exact ages were not known.

### Resting metabolic rates

We used an open-flow push-through respirometry system (Sable System, Las Vegas, NV, USA) to measure rates of CO<sub>2</sub> (VCO<sub>2</sub>) production of animals at rest. We used a similar protocol as described in Louppe *et al.* (2018). We also measured rates of O<sub>2</sub> (VO<sub>2</sub>) consumption, but given the age of the fuel cell, the data were not accurate enough to be used. During trials, individuals were placed gently in 350-mL cylindrical chambers (RC-2 Sable Systems). Air was pumped with a Dual Pump System (PP2; Sable Systems) from a Dewpoint Generator (DG-4; Sable System) allowing to humidify the air before entering to the system, thus preventing stress and desiccation of the animals (relative rate of humidity set at 95%). Although *X. allofraseri* is a predominantly aquatic species, it uses pulmonary respiration. The airflow was split into eight paths and then passed through the Flow Measurement System (FB8; Sable Systems) to control the flow rate which was set at

around 100 (±15) mL min<sup>-1</sup>. The controlled air then transited the eight chambers, seven occupied by an animal and the last one remaining empty as a baseline. The Flow Multiplexer (MUX; Sable Systems) allowed to sample one chamber's air stream and directed it to the detector of the Field Metabolic System (FMS; Sable Systems) to record the water vapor, barometric pressure, flow rate, ambient temperature, oxygen, and carbon dioxide. Each cycle was composed of 2 min of baseline, 5 min for each chamber sequentially, and then again 3 min of baseline. A total of eight cycles were recorded during the morning and afternoon. Mean values of temperature and carbon dioxide volume produced (VCO<sub>2</sub>) were calculated for each individual and for each cycle. The minimum amount of carbon dioxide produced (VCO<sub>2\_min</sub>) per individual across the eight cycles was used in further statistical analyses (i.e. resting metabolic rate). Ambient temperature experimented by each individual varied slightly during experimentation and increased by 0.33°C on average along the eight cycles.

### Body size measurements

Snout–vent length (SVL), measured as the distance from the posterior margin of the cloaca to the tip of the snout), was measured for all individuals prior to experimentation using a digital caliper (Mitutoyo; precision 0.01 mm). We also analyzed data of body mass collected several weeks prior to this study. SVL is highly correlated to body mass in *X. allofraseri* ( $r = 0.95$ ,  $P$ -value < 0.001; Fig. 1). Therefore, SVL can be used as a good proxy for overall size.

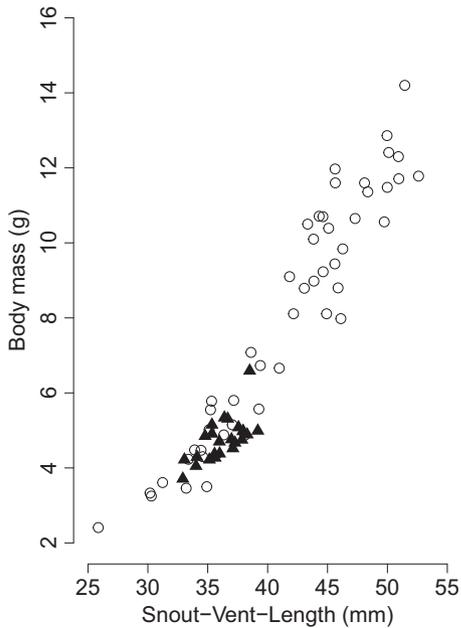
### Statistical analyses

All statistical analyses were performed in the R software (R Core Team, 2018). The first two cycles were discarded because animals moved and as such measurements did not represent resting metabolism (see Supporting Information, Figure S1). Individuals with missing values for at least one of explanatory variables were discarded prior to analysis, given a final dataset of 73 individuals (24 males and 49 females). We explained variation in VCO<sub>2\_min</sub> by sex, ambient temperature, and body size (body mass or SVL) in a linear model. Indeed, variation in the ambient temperature observed between chambers due to experimentation can be elevated, and therefore, we took this effect into account in the model (min = 19.39°C, max = 22.42°C, mean = 20.89°C, SD = 0.78°C). Because residuals did not meet normality assumptions, we log-transformed the response variables. The addition of quadratic terms to account for possible non-linear relationships between the response variables and body size or temperature did not improve the model fit and thus were not retained in the final model.

## Results

### Resting metabolic rates

Using SVL as body size factor, VCO<sub>2\_min</sub> was explained by all explanatory variables. Yet, the model accounted for only half



**Figure 1** Body mass (g) as a function of snout–vent length (SVL) (mm) in mature males (black triangles) and females (open circles).

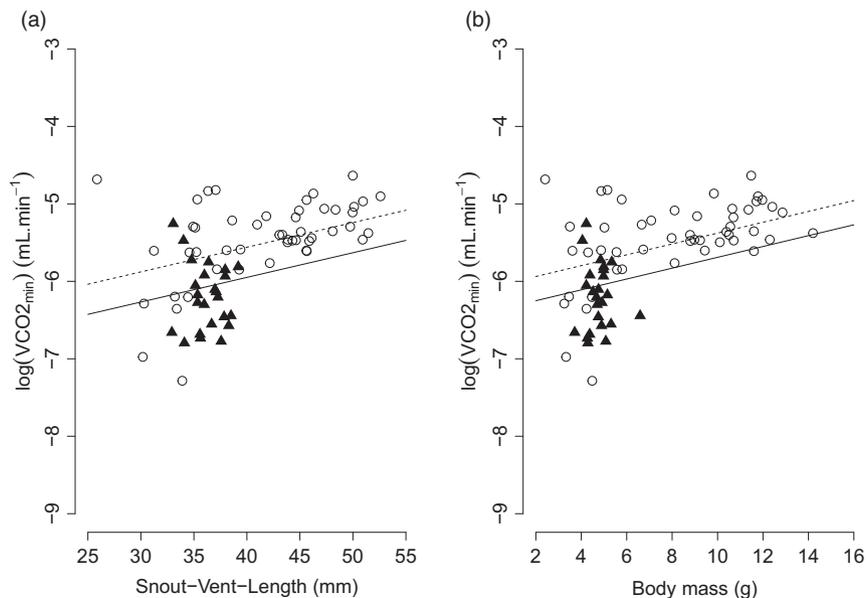
of the variation in  $\log(\text{VCO}_{2\text{min}})$  ( $r^2_{\text{adjusted}} = 0.49$ ), which indicates that other variables explain a considerable amount of variation in resting metabolic rate. Interestingly, after controlling for SVL ( $t$ -value = 3.35,  $P$ -value = 0.0013), and ambient temperature effects ( $t$ -value = 2.53,  $P$ -value = 0.014) in the

regression model, females had on average about a 50% higher  $\text{VCO}_2$  production than males (mean<sub>male</sub> = 0.0022 mL  $\text{CO}_2 \text{ min}^{-1}$ , mean<sub>female</sub> = 0.0048 mL  $\text{CO}_2 \text{ min}^{-1}$ ;  $t$ -value =  $-2.8$ ,  $P$ -value = 0.006, Fig. 2). To test if the effect of body size on  $\text{VCO}_2$  production varies between sexes, we added an interaction term between sex and body size, and this interaction was not significant (Sex\*SVL,  $t$ -value =  $-1.5$ ,  $P$ -value = 0.14).

We obtained similar result using body mass instead of SVL in the linear model. Variation in  $\text{VCO}_{2\text{min}}$  was significantly explained by ambient temperature ( $t$ -value = 2.80,  $P$ -value = 0.007), body mass ( $t$ -value = 3.59,  $P$ -value < 0.001), and by sex ( $t$ -value =  $-2.2$ ,  $P$ -value = 0.033).

## Discussion

As expected, RMR for both males and females was positively correlated with body size (both mass and SVL) and ambient temperature, based on the  $\text{VCO}_{2\text{min}}$  analysis. Our study also revealed that, after taking into account differences in body size, females had about a 50% higher production of  $\text{VCO}_2$  than males. The direction of this sexual dimorphism for RMR is similar to the results observed for salamanders (Finkler *et al.*, 2003), mammals (bank voles, Boratynski & Koteja, 2010), and insects (desert fleas, Krasnov *et al.*, 2004), but different from results on other ectotherms (toads, Finkler *et al.*, 2014; lizards, Garland and Else, 1987; tarantula, Shillington, 2005; wasp, Tomlinson & Phillips, 2015). Yet, our result is similar to those of a previous study on the frog *Xenopus laevis*, where females exhibited higher standard metabolic rate than males in populations at the invasion front compared to those from the center of the range (Louppe *et al.*, 2018).



**Figure 2** Log values of  $\text{VCO}_{2\text{min}}$  produced ( $\text{mL min}^{-1}$ ) as a function of (a) snout–vent length (SVL) and (b) body mass, in males (black triangles) and females (open circles). Full and dashed lines correspond to the regression lines for males and females, respectively. The effects of sex, body size, and ambient temperature were significant in explaining  $\log(\text{VCO}_{2\text{min}})$ .

Evolutionary explanations for sexual dimorphism in resting metabolism are still under debate. On the one hand, it has been suggested that individuals with low RMR can allocate more unused energy toward fitness-enhancing processes like reproduction – called the ‘compensation’ hypothesis (Nilsson, 2002), thus giving a selective advantage of a lower cost of self-maintenance. On the other hand, it has been argued that individuals with higher RMR may have the capacity to eat more food and convert it into usable energy at a higher rate, potentially resulting in a positive impact on reproductive output – called ‘the increased intake’ hypothesis (Nilsson, 2002). In the house sparrow, *P. domesticus*, female birds with higher basal metabolic rates show a reduced offspring recruitment, which supports the ‘compensation’ hypothesis (Rønning *et al.*, 2016). Whether one or the other hypothesis better explains our results remains to be explored in greater detail. Yet, there is clear evidence that male amphibians can incur very high metabolic costs while vocalizing during the mating season (Hillman *et al.*, 2008; Ophir *et al.*, 2010). Therefore, a lower RMR in males compared to females at similar body size could be selectively advantageous as unallocated energy may be devoted to costly mate finding or mating, thus supporting the ‘compensation hypothesis’. Indeed, in many frogs including *Xenopus* frogs males move more than females on average (e.g. Trochet *et al.*, 2016; Courant *et al.*, 2019) and as such a lower resting metabolism may allow them to allocate more energy to movement and mate finding.

The mechanisms underlying differences in resting metabolic rates between sexes in *X. allofraseri* frogs could be related to the differences in the proportions of different tissue types in both sexes, particularly in relation to gamete production. A study across numerous species, including amphibians, showed that males and females devoted about 0.1 and 300%, respectively, of the energy used for standard metabolism to the production of gamete biomass. Therefore, the cost of egg production is more than three orders of magnitude higher than the cost of sperm production (Hayward & Gillooly, 2011). In amphibians in particular, germ cells differentiate into oocytes continuously throughout life (Slack, 2009). During the growth phase, the oocytes acquire a large amount of yolk proteins produced by the female’s liver (Slack, 2009). As shown in adult *Rana temporaria*, the cortex of a fully developed ovary is composed almost entirely of diplotene oocytes belonging to all developmental classes (previtellogenic and vitellogenic phases) (Ogielska *et al.*, 2010). Therefore, the metabolic cost of such reproductive structures in females likely increases the metabolic cost and may result in a higher production of carbon dioxide. Future studies evaluating specific metabolic rates of major organs and tissues, particularly those related to reproduction, using mechanistic models of resting energy expenditure (Wang *et al.*, 2010), would provide further insights into the proximate causes of sexual dimorphism in RMR in *X. allofraseri*, and more generally in amphibians.

Regrettably, our measurements of  $VO_2$  were not sufficiently accurate to consider them in this study, and as such, we could not determine the respiratory quotient ( $RQ = VCO_2/VO_2$ ). This index usually serves as an indicator of the metabolic substrates (lipids, proteins, or carbohydrates) use by tissues. Living cells require ATP for energy, which is produced through the

anaerobic and aerobic pathways. The anaerobic pathway, through glycolysis located in the cytosol, produces a lower amount of ATP but at a much faster rate, compatible with a fast need for energy. On the other hand, endurance requires a prolonged and higher amount of ATP that is provided by the aerobic pathway and particularly by oxidative phosphorylation (i.e. degradation of lipids) in the mitochondrial respiratory chain. Information about the respiratory quotient would permit to highlight if, as for the  $CO_2$  production, a sexual dimorphism exists in the use of energy substrate at rest. For instance, men and women after fasting show differences in the use of lipids but not carbohydrates, and this gender-based metabolic differences increase with more prolonged fasting (Hedrington & Davis, 2015). It has been suggested that this observed difference in lipid mobilization is due to sexually dimorphic changes in anatomic fat distribution, men having a higher distribution of visceral fat and women having greater lower body and subcutaneous fat. In reed frogs, *Hyperolius marmoratus*, males not fed for a week had significantly lower RQ’s during calling than recently fed males and thus utilized approximately twice as much lipid to fuel calling (Grafe, 1996). It would be interesting to investigate this index in the future to more fully understand differences between males and females in resting metabolism and its possible consequences in terms of fitness.

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## Author contributions

AH, C.B, MV, and VD conceived the study; MV and CM did the measurements; VD analyzed the data and wrote a draft of the paper and all authors read and corrected the final version of the paper.

## Data availability

All data generated and analyzed during this study are available in the supplementary material.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Set of measurements (i.e. cycle) of  $VCO_{2\min}$  production values recorded during the experiments.