Sexually dimorphic growth and maturity in captive mountain chicken frogs *Leptodactylus fallax*

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ABSTRACT - Sexual dimorphism is commonly encountered in amphibians. Its presence and its ontogeny in a given species has implications for ecology, conservation and captive husbandry. We monitored changes in length and mass of captive mountain chicken frogs *Leptodactylus fallax*. Initially, sexes were no different in snout-vent length or mass, but by about 17 months after metamorphosis females became significantly larger and heavier than males. Diverging growth trajectories between male and female frogs appeared to coincide with the development of secondary sexual characters in males, indicating sexual maturity, while female frogs did not produce nests until the following breeding season, suggesting later reproductive maturity. The absence of similar dimorphism in other large leptodactylids suggests that its presence in *L. fallax* may be linked to its unique and energy-intense reproductive strategy, which involves extended maternal provisioning of their young.

INTRODUCTION

Sexual dimorphism in size is common among anuran amphibians and often reflects asymmetric investment in reproduction (see review in Wells, 2007). Such dimorphism may result from unequal growth rates between juvenile anurans, and/or from unequal durations of rapid growth before asymptotic growth rates are achieved (Miaud et al., 1999; Gramapurohit et al., 2005; Zhang & Lu, 2013; Otero et al., 2017). Sexual size dimorphism is likely driven by differing reproductive selective pressures linked to relative investment in gametes and competition for access to mates (Zhang & Lu, 2013). Asymmetric growth rates between males and females could inform population management decisions made by captive husbandry practitioners working with anurans in captive conditions by providing clues as to the sex of animals.

Mountain chicken frogs *Leptodactylus fallax* are Critically Endangered and dependent, in part, on captive populations for survival (IUCN SSC ASG, 2017). The species breeds seasonally with female frogs investing heavily in energetically costly maternal care (Gibson & Buley, 2004). Species biology and captive management requirements, both important in conservation strategies, have been heavily informed by data collection from captive populations (e.g. Gibson & Buley, 2004; Tapley et al., 2015; Jayson et al., 2018a&b; Michaels et al., 2021). Much of this information would be difficult or impossible to collect from wild animals due to the small or extinct status of populations, inaccessibility of some remaining populations, and the secretive nature of the species.

We monitored growth in mass and length of captive bred *L. fallax* in a conservation breeding facility over 397 days and used these data to investigate patterns of growth between metamorphosis and sexual maturity.

METHODS

Study animals and husbandry

The study used 12 full sibling, juvenile *L. fallax* captive bred at ZSL London Zoo as part of the Mountain Chicken Recovery Programme, hatched 12 May 2016 and metamorphosed 15–22 July 2016. The frogs were the offspring of a three-year-old captive bred female and a four-year-old captive bred male and were the same animals used as part of a study on dietary calcium provision (Michaels et al., 2021). Animals were exposed to a diurnal thermal gradient of 23–34 °C, a nocturnal gradient of 21–23 °C, an Ultraviolet Index (UVI) of 0–3 correlated with heat and photoperiod of 12:12; full details of husbandry can be found in Michaels et al. (2021). Individuals were identified through Visible Implant Elastomertagging (Nauwelaerts et al., 2000) and photographic IDs. Animals were fed a varied diet of invertebrates following Jayson et al. (2018a). Feeds were supplemented with a 1:1 by weight mix of Vetark Nutrobal® (VETARK Professional, Winchester, UK) and powdered calcium carbonate (product code P0302, Cambridge Commodities, Cambridge, UK), which was dusted onto prey items (Michaels et al., 2014) in the regimens described by Michaels et al. (2021). Frogs consistently held an acceptable body condition of between three and four on the body condition scoring scale developed by Jayson et al. (2018b).

Data collection

As part of routine health checks on all juvenile individuals of this species, frogs were captured by hand at approximately three months of age and weighed using digital balances (Salter Housewares, UK) approximately every 14 days between 4 November 2016 (day 0) and day 168, then a final measurement taken on day 397 following a hiatus in measurements caused by insufficient staffing resources to collect data frequently.

Snout-vent lengths (SVLs) were measured using digital callipers (Transcat, USA) to the nearest millimetre on days 0, 88, 168 and 397. This resulted in four SVL measurements and thirteen mass measurements. Sexual maturity in males was determined based on the development of nuptial spurs (keratinised or otherwise) (Jameson et al., 2019) and the date at which keratinised spurs were first detected was noted to indicate sexual maturity. Females were regarded as being at sexual maturity once they had produced a foam nest. The majority of frogs were exported to other collections at the end of the study, and before first reproduction in females, but for animals that remained in the ZSL collection, the date of first nest production was also recorded.

Statistical analysis

We did not use the von Bertalanffy growth function to model growth as frogs in our data set had not reached asymptotic growth and it was impossible to collect more data (frogs were exported to other collections) (Allen, 1969; Miaud et al., 1999). Instead, we used the *lme4* package in RStudio to build generalised linear mixed models (Bates et al., 2015), fitting the model Response Variable (SVL or Mass) = time + sex + time * sex, fitting frog individual as a random factor to control for repeated measures. We confirmed that model assumptions were met through visual inspection of residuals via the ggResidpanel function in R (Goode & Rey, 2019). The anova() function of the stats package (R CoreTeam, 2021) was then used to test the main effects and interactions sequentially. Data were tested for normality using a Shapiro Wilks test and one-way ANOVAs (stats package; R Core Team, 2021) were then used to compare measures between sexes at the start of the study and at the penultimate and final measurements.

RESULTS

Sex ratio, nuptial spur development and nesting

Of the twelve study frogs, seven were sexed as female and five as male. Keratinised nuptial spurs were first observed in male frogs between days 141 and 155, c. 8 months after metamorphosis. The first nests were produced by animals remaining (n = 4) in the collection from day 483, c. 20 months after metamorphosis.

Mass data

There was no difference in mass between sexes at the start of the study ($F_{1,16}$ = 0.32, p=0.6) (Fig. 1A). There was no significant effect of sex as a main effect ($F_{1,66.6}$=2.08, p=0.15). There was a significant effect of time ($F_{1,142}$=529.60, p<0.0001) and sex * time ($F_{1,142}$=64.5, p<0.0001). Model parameters are given in Table S1 (see Supplementary Material). Masses diverged between the penultimate and final measurements (Fig. 1A). Final measurement data were normally distributed (Shapiro-Wilk test, p=0.469) and, at the final measurement, there was significant difference between sexes ($F_{1,16}$=53.564, p<0.001). Sexual dimorphism index (SDI; Lovich, J.E. & Gibbons, 1992) at this point was 1.38; mean (±SD) mass was 382±24.1 g for females and 276.6±25.4 g for males.
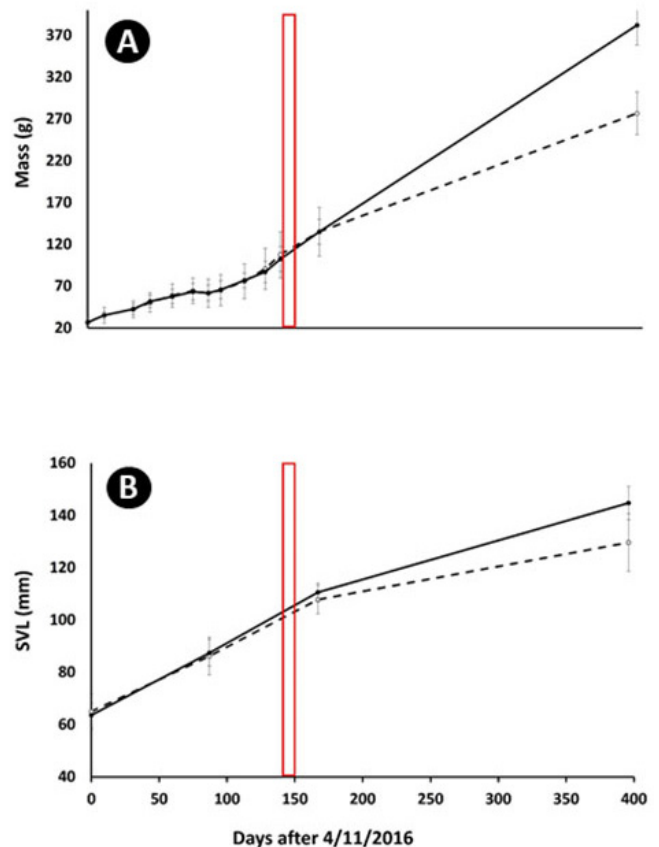

Figure 1. Growth of juvenile mountain chicken frogs in captivity - **A.** Changes in mass, **B.** Changes in snout-to-vent-length. The graphs show male (broken line; n=5) and female (solid line; n=7) frogs. The red box indicates the period over which keratinised nuptial spurs were first observed in males.

SVL data

There was no difference between sexes in SVL at the start of the study ($F_{1,16}$=0.29, p=0.6) (Fig. 1B). There was no significant effect of sex as a main effect ($F_{1,24.6}$=0.7, p=0.41). There was a significant effect of time ($F_{1,34}$=529.6, p<0.0001) and sex * time ($F_{1,34}$=7.45, p=0.01). Model parameters are given in Table S1. SVL diverged between sexes at between the penultimate and final measurements (Fig. 1B); final measurement data were normally distributed (Shapiro-Wilk test, p=0.416) and, at the final measurement, there was significant difference between sexes ($F_{1,16}$=9.119, p=0.013). SDI at this point was 1.11; mean (±SD) SVL was 144±6.4 mm for females and 129±11.0 mm for males.

DISCUSSION

Our analyses demonstrated that although sexes were initially no different in SVL or mass, growth trajectories varied such that females became significantly larger and heavier than males by the final measurement at approximately 17 months after metamorphosis. Our models were a good fit with high R^2 values and relatively small confidence intervals around estimates; conditional:marginal R^2 ratios were close to 1:1

for both models and random effect standard deviation was low, suggesting the effect of individual separate from sex was small. Sexual dimorphism in either measure of growth was not apparent at 8 months or before, but was present at 17 months when the next measurement was taken. Unfortunately, no data were available during this period to detect at exactly which point growth trajectories became significantly different. However, keratinised nuptial spurs, a characteristic of sexual maturity, were developed in males at 8–8.5 months post metamorphosis. This coincided slightly before the start of the data gap and it is most likely that growth rates became sexually dimorphic shortly after this point. Our data regarding male sexual maturity at c. 8 months for males and female first reproduction at c. 20 months is congruent with anecdotal evidence from other captive collections (Jameson et al., 2019), but is the first record of this information in the scientific literature. This represents rapid onset of sexual maturity given the size of the species and aligns with the suggestion that the species is short lived but with rapid growth to maturity in the wild (seven years maximum longevity, Guarino et al., 2014) as well as in captivity (longevity 6–12 years, Jameson et al., 2019). These findings are congruent with those of other studies on leptodactylid frogs. Both sexes of *Leptodactylus labyrinthicus* are considered sexually mature at 8 months of age (Silva et al., 2005). Sexual maturity is attained within the first year in *Leptodactylus latrans* with more males attaining sexual maturity within the first year than females (López et al., 2015).

Sexual size dimorphism is likely due to different investment in reproduction (Zhang & Lu, 2013). Typically, large bodied *Leptodactylus* species do not exhibit sexual size dimorphism, or if they do then females are smaller than the males (Camurugi et al., 2017). The SDI in the study frogs was substantial, with females being on average 10 % larger than males and 40 % heavier. There was, however, overlap between sexes such that some males were larger than some females. In *L. fallax*, although males compete to gain access to females and to hold suitable nesting sites (King et al., 2005), females must produce large clutches of eggs and feeder eggs (10,000–25,000 eggs per clutch) and then guard and feed young which results in their own reduced food intake (Gibson & Buley, 2004; Jameson et al., 2019). Larger body size is likely important in meeting the energetic demands of this reproductive mode.

Sexual dimorphism in size has been detected in numerous anuran taxa; larger adult females is a common pattern (Zhang & Lu, 2013). Although in many cases this is due to more rapid growth rates alone (e.g. Ma & Lu 2009; Sarasola-Puente et al., 2011; Otero et al., 2017), it is often driven by delayed sexual maturity and therefore delayed asymptotic growth in females, (Gramapurohit et al., 2005), or due to a combination of growth rates and delayed maturation (Miaud et al., 1999). In a study looking across anuran lineages, Zhang & Lu (2013) found that the typical trend is for lower growth rates but longer longevity in females, leading to eventually larger size in females. In the frogs in this study, diverging growth trajectories appear to coincide with the development of secondary sexual characters in males, indicating sexual maturity, while female frogs did not produce nests until the following breeding season, suggesting later reproductive maturity. Female frogs may have become

sexually mature at the same point, but waited until the next environmentally-triggered breeding season to produce clutches. The development of keratinised spurs, however, coincided with the very beginning of the breeding period that year, and yet females did not produce spawn until the following season, a year later. This suggests that females were not sexually mature at the same time as males and reached this point later, following the first breeding season of male siblings. This coincidence suggests that earlier sexual maturity in male *L. fallax* may at least partially explain the patterns we detected. We did not see evidence for relatively slow female growth rates, so these results only partly align with the wider anuran trends reported by Zhang & Lu (2013). However, this may be explained by the relatively short lifespan and large size of this species (Guarino et al., 2014). Selective pressure for females to adopt a strategy of slower growth protracted over a longer period may not be viable, leading instead to the development of larger female size under accelerated growth at sexual maturity in order to achieve the same fecundity benefit (Zhang et al., 2013).

Unfortunately, management of the wider captive population of this species required animals from this study to be moved to other holders and we were unable to continue following this cohort's growth in order to establish the point of asymptotic growth in these animals. Further research in the captive population should seek to track animal growth over longer periods to better understand this aspect of biology, including data covering a longer period of development after sexual maturity, and potentially from a broader genetic background within the species (all focal animals presented here were full siblings).

Although our data contribute to the wider understanding of the biology of this species, the coincidence of sexual dimorphism in size with the acquisition of binary secondary sex characters, such as spurs, limits its utility in a practical context in terms of sexing animals. However, differing growth rates between sexes from the onset of maturity may suggest differing nutritional requirements between sexes at this point. Given the tendency of this species to develop pathologies linked to calcium metabolism (Tapley et al., 2014; Jayson et al., 2018a; Michaels et al., 2021), it may be particularly important to provide this mineral, and others, with this in mind.

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