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SYMPOSIUM

The Effect of Pregnancy On Metabolic Scaling and Population Energy Demand in the Viviparous Fish *Gambusia affinis*

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Synopsis Metabolism is a fundamental attribute of all organisms that influences how species affect and are affected by their natural environment. Differences between sexes in ectothermic species may substantially alter metabolic scaling patterns, particularly in viviparous or live-bearing species where females must support their basal metabolic costs and that of their embryos. Indeed, if pregnancy is associated with marked increases in metabolic demand and alters scaling patterns between sexes, this could in turn interact with natural sex ratio variation in nature to affect population-level energy demand. Here, we aimed to understand how sex and pregnancy influence metabolic scaling and how differences between sexes affect energy demand in *Gambusia affinis* (Western mosquitofish). Using the same method, we measured routine metabolic rate in the field on reproductively active fish and in the laboratory on virgin fish. Our data suggest that changes in energy expenditure related to pregnancy may lead to steeper scaling coefficients in females ($b = 0.750$) compared to males ($b = 0.595$). In contrast, virgin females and males had similar scaling coefficients, suggesting negligible sex differences in metabolic costs in reproductively inactive fish. Further, our data suggest that incorporating sex differences in allometric scaling may alter population-level energy demand by as much as 20–28%, with the most pronounced changes apparent in male-biased populations due to the lower scaling coefficient of males. Overall, our data suggest that differences in energy investment in reproduction between sexes driven by pregnancy may alter allometric scaling and population-level energy demand.

Introduction

Metabolic rate is a fundamental component of all organisms that influences how species affect their natural environment and constrains the energy available for reproduction (Brown et al. 2004). Metabolic rates for many species (mammals, fish, birds) often scale with body mass following $\frac{3}{4}$ power scaling (Kleiber 1975; West et al. 1997; Brown et al. 2004). A predominant explanation for $\frac{3}{4}$ power scaling is fractal network theory, which champions that transport networks constrain metabolic rates at larger body sizes (West et al. 1997, 1999; Harrison et al. 2022). Despite being widespread, $\frac{3}{4}$ power scaling is not universal within or across species (Glazier 2005). There is substantial variation in scaling parameters for body size, particularly among divergent phylogenetic taxa (Uyeda et al. 2017). Variation in metabolic scaling parameters across and within some species has been linked to differences in body size ranges (Glazier 2005), temperature (Dell et al. 2011; Moffett et al. 2018), and sex, particularly in dimorphic species (Shillington and Peterson 2002; Shillington 2005; Strauss and Reinhold 2010; Messamah et al. 2017). For example, in many species, males show sexually exaggerated traits (e.g., antlers, horns) to increase reproductive fitness, and they often show relatively steep allometric scaling (>1)

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(Kodric-Brown et al. 2006; Eberhard et al. 2018; Somjee et al. 2022).

Reproductive differences between sexes may influence allometric scaling, though few studies have assessed these effects, particularly in ectotherms (but see Srean et al. 2016). Most studies comparing metabolic rates between sexes have focused on mass-specific oxygen requirements rather than scaling coefficients (Weldon et al. 2013; Tomlinson and Phillips 2015; Kraskura et al. 2021). Such an approach allows for misinterpretation because mass-specific metabolic rates systematically underestimate metabolic rate and may lead to misunderstandings about differences in metabolic rate across or within species (Hayes 2001; Müller et al. 2021). For example, female Drosophilidae have lower mass-specific metabolic rates than males but steeper scaling coefficients (Messamah et al. 2017). Consequently, the use of mass-specific rates may lead to an inaccurate application of metabolic patterns, such as in ecological modeling. Further, error associated with mass-specific interpretations of metabolic rate is predicted to increase with body mass, leading to erroneous interpretations for dimorphic species. Accordingly, a clearer understanding of how metabolism scales with body size between sexes is required to characterize population-level energetic demand accurately.

Sex likely influences organismal energetic demand through embryo or sperm production costs. Generally, females invest an average of 3.5x more energy in gamete production than males, though variation in female gamete investment ranges from 0.1–300% of standard metabolic costs (Hayward and Gillooly 2011). Viviparous (live-bearing) fishes are a promising study system to examine such effects because differences between sexes may be pronounced (Timmerman and Chapman 2003). While viviparity offers advantages to offspring in the form of thermoregulation, protection, dispersal, and nutrient provisioning, females must also support metabolic rates of their embryos and supply nutrients to the embryos via the yolk and direct supply (Goodwin et al. 2002). When females reach maturity and become pregnant, the additive costs of their standard metabolic rate (SMR) and that of their embryo's metabolic rate (including nutrient provisioning) may increase allometric scaling exponents as energy requirements increase. For example, before pregnancy, females must support only their own metabolic costs, whereas, during pregnancy, females must support the metabolic and nutrient provisioning costs of their embryos in addition to their metabolic costs, leading to a disproportional increase in metabolic demand at larger body sizes which may alter allometric scaling slopes.

Here, we examine differences in the allometric scaling of metabolism between sexes in a globally invasive freshwater fish species, *Gambusia affinis* (hereafter *Gambusia*). *Gambusia* were collected from populations spanning a wide thermal gradient to examine population-level differences. We measured routine metabolic rate (RMR) (average amount of energy expended when the fish is undergoing normal behaviors) on both pregnant fish measured in the field and virgin fish measured in the laboratory to determine how reproduction influences allometric scaling. Our key questions are: (1) Does allometric scaling differ between males and females? (2) Does pregnancy influence metabolic scaling? And (3) Does accounting for sex-specific scaling alter population-level estimates of energetic demand? We hypothesized that pregnancy in *Gambusia* will be associated with higher energetic costs due to the internal development and provisioning of live young, resulting in steeper scaling coefficients.

Methods

Study species and system

Gambusia are small (males <3.5 cm; females <6 cm), live-bearing, sexually dimorphic fish found globally across a wide range of environmental conditions (e.g., salinity, temperature, pH, turbidity) (Pyke 2008). In *Gambusia*, females exhibit indeterminate growth, whereas males virtually cease growth at maturity. *Gambusia* are an ideal species to address our aim because females are generally mated and become pregnant immediately upon reaching maturity (Pyke 2005). Pregnancy in mosquitofish involves both yolk (lecithotrophy) and nutrient contributions from mothers (matrotrophy), and mothers support the respiratory demands (O_2 and CO_2 exchange) of their offspring. Females can store sperm over the non-reproductive period, allowing them to reproduce as soon as environmental conditions become favorable. Mature females may have several continuous pregnancy cycles and deliver five or more clutches per year (Pyke 2008). In males, sperm are continuously generated throughout the breeding period. In most populations, the breeding season ranges from mid-spring to mid-autumn (Pyke 2005). *Gambusia* does not show superfetation (common in other poeciliids); consequently, only one brood develops in the ovary at any time, and the embryos are at a similar developmental stage (Krumholz 1948).

Our study on populations of *Gambusia* is from geothermal springs that span a wide thermal gradient in California and in the North Island of New Zealand. *Gambusia* in New Zealand and California are derived from populations in Texas, USA (Dill and Cordone 1997; Pyke 2008). *Gambusia* were introduced to New Zealand in the 1930s and California in the 1920s (McDowall 1978; Stockwell and Weeks 1999; Fryxell et al. 2021). In California, populations inhabit slow-flowing spring-fed ponds with barriers to dispersal; however, we cannot discount the possibility of fish movement among sites due to human activity. In New Zealand, populations inhabited slow-flowing, spring-fed streams, and four of the five systems are potentially open to fish movement.

RMR measurement

Gambusia metabolic rate was measured in the field (reproductively active) and the laboratory (virgin) using the same method. Data were collected from a series of studies for other purposes focused on metabolic patterns in the populations of *Gambusia* (Moffett et al. 2018; Fryxell et al. 2020; Benavente et al. 2022). We used virgin and reproductively active fish to determine how sex differences influence the allometric scaling of metabolism and whether this difference is due to differences in reproduction. In both virgin and reproductively active fish, we measured metabolic rate as RMR, in which we placed *Gambusia* into 40-mL acrylic respirometers for 20 min while measuring oxygen consumption. After a few minutes, we continuously measured each respirometer's dissolved oxygen concentration and temperature for 20 min using a FireSting four-channel oxygen logger with optical oxygen sensors (PyroScience, Aachen, Germany). We measured oxygen consumption in blank respirometers (water only) to control for microbial oxygen consumption.

Reproductively active fish

Metabolic rate was measured *in situ* in the field at 10 sites with a broad thermal gradient across California ($n = 5$ populations, source temperatures 19.2–29.9°C) and New Zealand ($n = 5$ populations, source temperatures 24.8–36°C). Moffett et al. (2018) detail the full collection protocol. We assume mature females were reproductively active (*i.e.,* cycling through pregnancies) at the time of metabolic rate measurement as sexes co-occurred, and sampling was carried out in summer when *Gambusia* are at their reproductive peak (Pyke 2005). For example, a field survey in California revealed that 86.3% of females were visibly pregnant in summer. Similarly, in New Zealand, 85.1% of fish were visibly pregnant in summer (Fryxell et al. 2020; Moffett et al., unpublished data), and in both locations, more may have been pregnant, but their pregnancy may not have been visible (*i.e.,* they may have been in the early stages of pregnancy). At each site, we captured 50 fish spanning a wide size range (6–336 mg dry mass) for the measurement of RMR (see *RMR measurement)*. We held the respirometers in a submerged 50-L clear container of site water during runs to maintain ambient water temperature. *Gambusia* were captured and held for 20-min in advance of each run to establish uniform holding times and conditions before measurements. After metabolic rate measurements, we euthanized fish using clove oil in New Zealand and an overdose of MS-222 in California. Fish were then measured for mass, length, and sex, then dried at 60°C for 48 h and reweighed for dry mass. Fish collection, rearing, and metabolic measurement were approved by the University of California Santa Cruz (protocols PALKE-1311–2) and The University of Auckland animal ethics committees (Ref. 001089). In California, fish collection was also approved by the local wildlife agency (CADFW permit SC-12726).

Virgin fish

Gambusia from six populations in California were common-reared in a controlled environment for two generations. Fryxell et al. (2020) detail the entire collection and rearing protocols. Briefly, field-caught *Gambusia* were first reared at a greenhouse at the University of California Santa Cruz at 26 ± 1°C, an intermediate temperature of the collection sites for one generation (F1). Once second-generation fish (F2) were born, we immediately moved them into controlled environment rooms, where we reared them at four temperatures (23 ± 0.5, 26 ± 0.5, 30 ± 0.5, and 32 ± 0.5°C). F2 fish were assigned a unique ID number and reared individually inside mesh cylinders with a petri dish bottom and an open-top (250 μm mesh, 7 cm diameter, 20 cm height). Each holding one fish, cylinders were sunk upright into 100 L plastic tubs (91 × 61 × 20 cm) filled with off-gassed city water. F2 fish had no contact with conspecifics during this common rearing stage, though chemical cues from both sexes would have been present in the aquaria. We starved the *Gambusia* for 16-hours before RMR measurements to ensure they were in a non-digestive state. Following metabolic rate measurements, we euthanized the fish with an overdose of MS-222. Fish were then measured for mass, length, and sex, dried at 60°C for 48 h and reweighed for dry mass. Fish collection was approved by the University of California Santa Cruz animal ethics committee (PALKE-1311 and PALKE-1801) and by the local wildlife agency (CADFW permit SC-12752).

Statistical analysis

Reproductively active fish and virgin fish

We removed juveniles (individuals that could not be accurately sexed) data from the analysis, so all individuals in our dataset were sexually mature at the time of metabolic rate measurement. We determined the allometric relationship (i.e., slope) between body size and RMR for both sexes in both virgin and reproductively active fish separately, using simple linear regression on log-transformed data. We do not analyze differences in intercept as we were interested in understanding how metabolic rate changes with increased body size. For each sex, we calculated the allometric relationships for reproductively active fish and virgin fish across all populations and separated by source population or laboratory acclimation temperature. In addition to calculating scaling coefficients across all individuals, we also calculated scaling coefficients using constrained datasets, where males and females had overlapping size ranges (Supplementary Fig. S1).

For analysis of reproductively active fish, we used a linear mixed-effects (LME) model to determine the effect of sex and body size on metabolic rate, with the country (NZ or USA) as a random effect. Our model was: *log10 metabolic rate* $\sim$ *log10 dry mass* $*$ *sex* $+$ *temperature* $+$ *(1* $|$ *Country).* Similarly, in our virgin data, we used an LME model to determine the effect of sex, source population, laboratory temperature, and body size on fish metabolic rate using the tank as a random effect. Our model was: *log10 metabolic rate* $\sim$ *log10 dry mass* $*$ *sex* $+$ *source temperature* $+$ *laboratory temperature* $+$ *(1* $|$ *tank number).* We removed the interaction between temperature and other factors in both models as this was not significant ($P > 0.05$). We used analysis of covariance (ANCOVA) to determine if female slopes were significantly different from male slopes. We used linear regression to determine if metabolic scaling coefficients varied with source temperature in reproductively active fish and laboratory acclimation temperature in virgin fish. All analyses were done in R version 4.1.0 (R Development Core Team 2021) using "lme4" v.1.1.28 (Kuznetsova et al. 2017) and calculated P values using "LmerTest" v.3.1–3 (Bates et al. 2015).

Scaling model

We explored how pregnancy influences metabolic scaling in female *Gambusia* by comparing models that assumed a female's metabolic rate was based on her mass alone and as a female whose mass was partitioned into her body and a brood of individual offspring. For our first model, which assumes no reproduction, the metabolic rate of virgin fish was estimated using the allometric scaling exponent of virgin females in our data set ($b = 0.876$, Fig. 1C). For our second model, we assumed fish were virgins until they reached 6 mg dry mass, or 0.78 mg on a $\log_{10}$ scale, after which we assumed they reproduce with a constant gonadosomatic index (GSI) of 15% across all size categories. A reproductive weight of 6 mg dry mass was based on previously collected size-distribution data, and we assumed a GSI of 15% based on fecundity data collected on *Gambusia* populations (Moffett et al. 2018; Fryxell et al. 2020). Metabolic rate in our second model was calculated as the metabolic rate of a virgin fish and the metabolic rate of embryos in the fish, assuming a constant scaling exponent of $b = 0.876$ for both embryos and adults when considered separately.

Population-level responses

Using data from our field populations (shown in Fig. 1A), we explored the importance of sex-specific allometric scaling in estimating population-level energetic demand. For each of our populations, which spanned sex ratios from 0.25 to 1.57 M/F, we estimated total population demand using combined and sex-specific scaling coefficients. For each population, we first assumed that each individual's metabolic rate followed a combined scaling coefficient ($b = 0.787$, field data derived from Fig. 1A) regardless of fish sex and calculated individual metabolic rate (B) as

$$B = b_0 M^b e^{-E/kT}, \qquad (1)$$

where b is the scaling exponent, E is the mean activation energy of metabolism, *b0* is a normalization constant, T is the temperature in kelvin, and k is Boltzmann's constant, and M is mass. The parameters *b0*, k, and T were the same for all individuals across populations. We used measurements of fish mass from field data for M. In the second model we used male and female scaling exponents from our field populations ($b = 0.595$ and 0.750, respectively, derived from Fig. 1A) to calculate population metabolic rate. We used the ratio of males and females, and their mass from our field data.

Results

Reproductively active fish

In reproductively active fish, allometric scaling depended on sex ($F_{1,332} = 8.82$, $P = 0.003$; Fig. 1A). Female fish had higher metabolic rates, but this difference between sexes was only apparent at larger body sizes (Fig. 1A, dry weight $>$ 1.4 mg). As such, the allometric scaling of metabolism was steeper for females ($b = 0.750$) than males ($b = 0.595$) with all populations combined (ANCOVA, $F_{1,200} = 57.2$, $P < 0.0001$). Within populations, the allometric scaling of metabolism was steeper for females compared to males in seven populations, the same in two populations, and was greater for males in one population (Fig. 1B). Metabolic rate was higher in populations with warmer temperatures ($F_{1,313} = 90.1$, $P < 0.0001$).

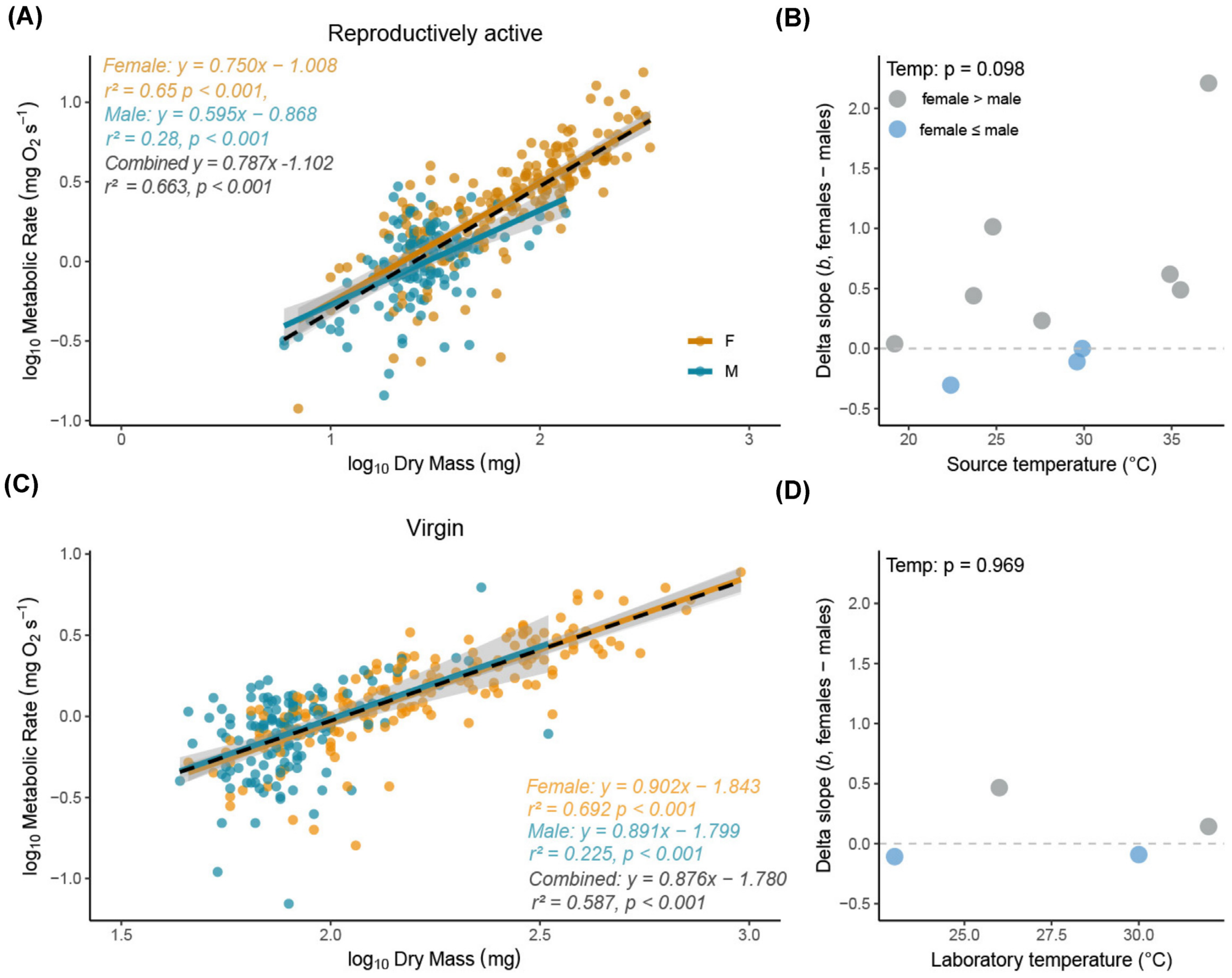

Fig. 1 Online-only. Allometric scaling of metabolic with sex rate across *Gambusia* populations as measured in the field (**A** and **B**) and common-reared in the laboratory (**C** and **D**). Metabolic rates measured in the field were from reproductively active individuals, whereas common-reared individuals were virgins reared from birth in isolation. Data shown in plots **A** and **C** are pooled across all source populations or laboratory temperatures, respectively. In plots **B** and **D**, we show the differences in female and male scaling coefficients when split by source populations or laboratory temperatures. Data above the dashed line at zero in plots **B** and **D** show that females have steeper scaling exponents than males, and the opposite is true for data below the dashed line.

However, we found no relationship between source population temperature and the allometric slopes of males ($F_{1,8} = 3.29$, $P = 0.107$) and females ($F_{1,8} = 0.720$, $P = 0.421$) or temperature and the difference in male and female slopes ($F_{1,8} = 3.518$, $P = 0.098$; Fig. 1B). When considering males and females that occupied overlapping size ranges, we also found a significant effect of sex ($F_{1,291} = 12.41$, $P = 0.0004$) (Supplementary Fig. S1), with steeper allometric slopes for females compared to males.

Virgin fish

We found no effect of sex ($F_{1,306} = 0.052$, $P = 0.821$) or source population ($F_{1,303} = 0.471$, $P = 0.751$) on metabolic rate in virgin fish (Fig. 1C). However, there was an effect of mass ($F_{1,298} = 106.626$, $P < 0.0001$) and laboratory rearing temperature ($F_{1,303} = 12.20$, $P = 0.002$) on metabolic rates, where metabolic rate increased in larger fish and warmer treatments. Allometric slopes of females were not different from those of males ($b = 0.902$ and 0.891 respectively) (ANCOVA, $F_{1,465} = 2.428$, $P = 0.089$). Across laboratory temperatures, the allometric scaling of metabolism was steeper for females compared to males at two out of four temperatures (26 and 32°C) (Fig. 1D). We found no relationship between laboratory temperature and the allometric slopes of males ($F_{1,2} = 0.107$, $P = 0.775$) and females ($F_{1,2} = 5.030$, $P = 0.154$), or temperature and the difference in male and female slopes ($F_{1,2} = 0.002$, $P = 0.969$; Fig. 1D). When considering males and females which occupied an overlapping size ranges, sex did not have a significant effect on metabolic rate ($F_{1,239} = 1.181$, $P = 0.278$).

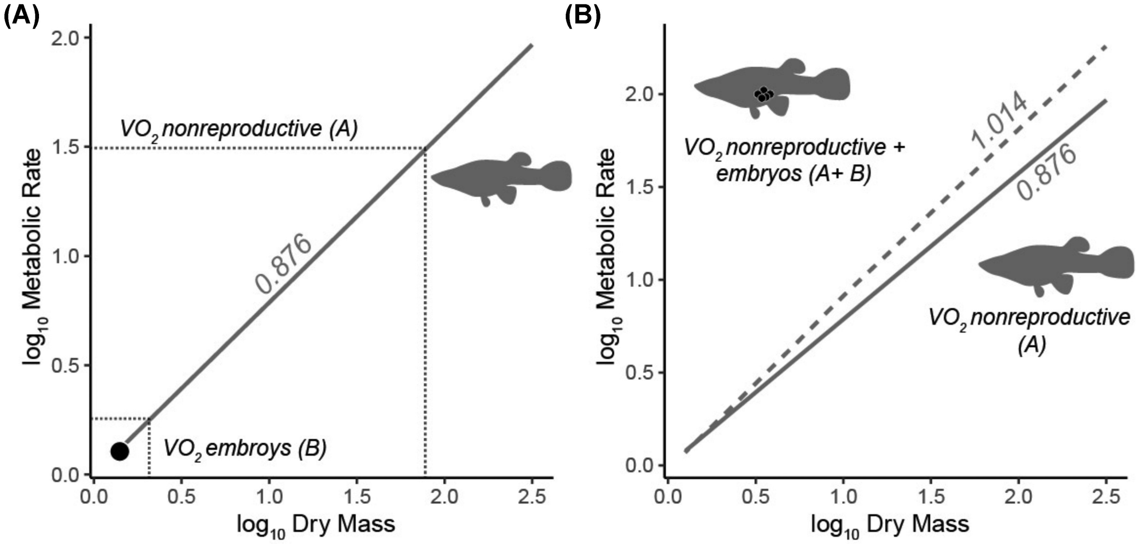

Fig. 2 Proposed model showing the change in metabolic oxygen requirements (VO$_2$) and allometric scaling of virgin and reproductively active female *Gambusia affinis*. In panel **A**, we show a theoretical relationship (based on Fig. 1C) between metabolic rate and mass, assuming embryo and adult metabolic rate follows the same scaling relationship. In panel **B**, embryo metabolic demand is added to a non-reproductive fish's metabolic demand to determine how reproduction influences scaling. We assume that fish remain virgins until they reach 6 mg dry mass (0.78 mg on the log$_{10}$ scale); after this point, individual embryos account for 15% of an individual's GSI.

Scaling model

In our model of differences in metabolism between pregnant and non-pregnant females, allometric scaling coefficients rose from 0.88 in virgin fish to 1.01 in pregnant fish, a 15% increase (Fig. 2). As such, our model predicts that the energetic cost of pregnancy leads to steeper scaling coefficients.

Population-level responses

Estimates of total population energy demand were consistently lower when sex-specific allometric scaling was used (Fig. 3). In particular, omitting sex led to estimates of population energy demand ranging from 20 to 28% higher than sex-specific models. Further, the difference in scaling between the combined and sex-specific models decreased with a higher proportion of males in the population, indicating that models which do not account for sex differences may overestimate population energetic demand in this species, particularly if sex ratios are male-biased.

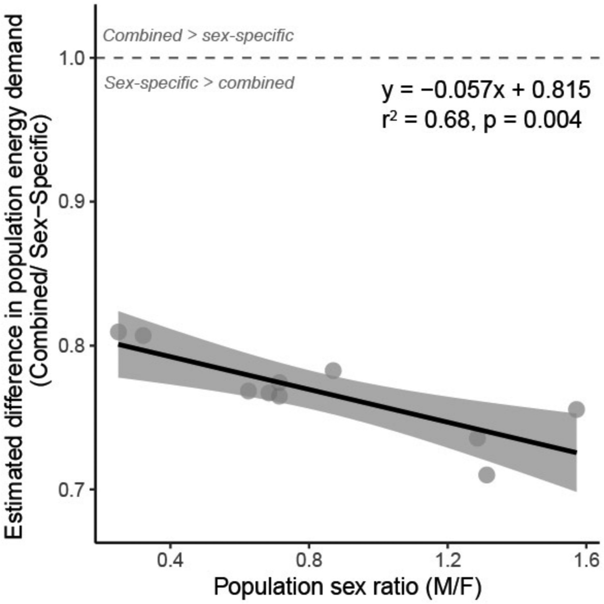

Fig. 3 Influence of population sex ratio on the ratio of population energy demand when considered using a combined scaling coefficient ($b = 0.787$) versus male and female coefficients ($b =$ m: 0.595, *f*: 0.750) using *Gambusia affinis* field data presented in Fig. 1. Data are fitted with a linear regression model.

Discussion

Live-bearing fish may incur substantial energetic costs due to reproduction (Timmerman and Chapman 2003; Hayward and Gillooly 2011), but it is unknown if differences in reproductive costs between sexes alter metabolic scaling and population-level energy demand. Our data show that changes in energy expenditure related to embryonic development and maintenance costs may lead to significant changes in the allometric scaling in metabolism, with steeper scaling coefficients for females compared to males when metabolic rate was measured on a reproductively active fish in the field. We show that virgin females and males had the same scaling coefficients when metabolic rate was measured in the laboratory. This similarity in scaling coefficients indicates that other differences (*e.g.*, behaviour, growth) between sexes have a negligible effect on the metabolic scaling of *Gambusia*. However, we do not know the relative contribution of other factors that may influence metabolic rate. Thus, the difference in metabolic scaling between males and females is likely due to female pregnancy. Further, we show that incorporating sex-specific scaling coefficients into population-level energetic demand estimates reduces whole-population energy demand in populations with greater male-to-female ratios.

Allometric scaling and reproductive mode

In reproductively active *Gambusia,* allometric slopes were steeper for females ($b = 0.750$) compared to males ($b = 0.595$), and this effect was common across most populations (Fig. 1). Though we do not have an explanation of the differences in slope across populations, variation may be linked to a combination of factors, including temperature, growth, diet, and fecundity (Moffett et al. 2018; Fryxell et al. 2020; Moffett et al. 2021; Moffett et al. 2022). Steeper scaling in reproductively active females is likely the result of the cost of provisioning nutrients to embryos and ongoing embryo maintenance costs (*e.g.,* respiration) (Timmerman and Chapman 2003; Schultz et al. 2008; Foucart et al. 2014; Mousavi et al. 2021). For example, in the viviparous northern death adder (*Acanthophis praelongus*), embryo metabolism accounted for 37%, and embryo maintenance costs accounted for 26% of the total metabolic rate (Schultz et al. 2008). *Gambusia* show lecithotrophy and matrotrophy, providing yolk and nutrients to embryos (Marsh-Matthews et al. 2001, 2005). In addition, during gestation, the mother must provide embryos with oxygen and discard any metabolic waste products. In some species, oxygen requirements increase in late pregnancy (Timmerman and Chapman 2003; Schultz et al. 2008; Foucart et al. 2014); though in *Gambusia holbrooki* there was no effect of the pregnancy stage on SMR (Seebacher et al. 2013). Regardless, the development of embryos due to provisioning and maintenance costs should increase metabolic demand, which our data show leads to differences in allometric scaling coefficients.

Embryo maintenance costs begin as soon as female *Gambusia* reach maturity (~17–22 mm) and their eggs are fertilized (Pyke 2005), leading to size-dependent changes in metabolic rate. Our model shows that organismal metabolism increased after embryo development, causing the allometric scaling coefficient to steepen (Fig. 2). An increase in the mass-specific metabolic rate of females during reproduction is common in viviparous ectothermic species (Cech et al. 1985; Weldon et al. 2013; Ladds et al. 2017; Lima-Santos et al. 2021). For example, in the African velvet worm (*Opisthopatus cinctipes*), females had higher mass-specific metabolic rates, thought to be due to the internal development of embryos (Weldon et al. 2013). Similarly, in the Tomodon snake (*Tomodon dorsatus*), the cost of reproductive maintenance in females was higher than the cost of vitellogenesis (yolk deposition), and there was no effect of reproductive effort on male metabolism (Lima-Santos et al. 2021). In contrast, Srean (2016) found no differences in RMR scaling coefficients between sexes in reproductively active *G. holbrooki,* though RMR was estimated using swim-tunnel measurements of maximum metabolic rate (MMR). The measurement method may influence the ability to detect sex-specific RMR differences due to differences in activity between sexes in the swim tunnel or due to physiological constraints in MMR measurements, which are much less flexible than RMR measurements (Sandblom et al. 2016). Together, these data suggest that reproduction in ectothermic viviparous species is likely to increase metabolic rate, leading to changes in allometric scaling. To extend on the findings in this paper, we suggest that future studies should aim to (1) report scaling coefficients, which allow for a greater understanding of the relationship between allometry and sex, and (2) consider sex and pregnancy as critical factors which may alter individual and population-level energy demand (discussed below).

In oviparous species, the most parsimonious explanation for sex differences in scaling between sexes where they exist is related to behavioral or morphology differences (*i.e.,* courtship, reproductive structures) (Beaupre et al. 1993; Rogowitz and Chappell 2000; Kolluru et al. 2004; Strauss and Reinhold 2010; Tomlinson and Phillips 2015; Somjee et al. 2018). The external development of embryos in oviparous species means lowered metabolic costs (*e.g.,* no embryo respiration costs) and fewer sex differences in scaling related to reproductive structures. As such, differences in metabolism between sexes are often species-specific. For example, in the Thynnine wasp (*Zaspilothynnus nigripes*), males have higher mass-specific metabolic rates due to differences in activity between sexes because females are flightless, whereas males fly in search of mates (Tomlinson and Phillips 2015). In another study, female scaling coefficients were lower than males in the lesser wax moth (*Achroia grisella*) due to the different mechanisms used to increase body size, females increase cell size, and males increase cell number (Strauss and Reinhold 2010). The commonality of differences in allometric scaling between sexes in viviparous and oviparous species indicates that sex should be incorporated when considering intra- and inter-specific metabolic scaling relationships across a range of species, particularly in species that show sexual dimorphism.

Allometric scaling and dimorphism

Different selective pressures likely drive variation in scaling exponents across sexes due to variation in somatic growth. Sexually dimorphic species, such as *Gambusia*, may exhibit differences in scaling due to differences in the proportional mass of metabolically demanding tissues between sexes. Our data show no difference in allometries between virgin males and females reared in captivity, indicating that differences in growth between sexes do not explain differences in scaling patterns. An increased proportion of metabolically inactive tissue in females (*e.g.,* lipids which have a high carbon content) may reduce metabolic demand (Walker and Irwin 2006). Indeed in *Gambusia*, body carbon is higher in females (average: 42.88%) when compared to males (average: 41.04%), which may have compensated for differences in somatic growth and contributed to the lack of difference in scaling between virgin males and females (Moffett et al. 2021). Despite the lack of difference in scaling between sexes in reproductively inactive *Gambusia*, differences in metabolic rate due to dimorphism are common in the literature and often increase reproductive fitness (Kotiaho 2001; Eberhard et al. 2018; Somjee et al. 2022). Regardless, our data suggest that differences in growth between sexes in *Gambusia* do not influence allometric scaling, though research to disentangle the behavioral, morphological, and physiological bases of sex differences in scaling may reveal trade-offs between sexes and reproductive stage. Indeed, future studies would benefit from measuring metabolic rate between sexes and reproductive modes across species in the same setting. Here, our use of field and laboratory fish constrained our ability to directly compare metabolic rates between datasets and exclude other factors that may have influenced metabolic scaling.

Population-level effects

Sex differences in allometric scaling might be predicted to lead to significant differences in population-level energy demand, particularly if populations are male- or female-biased. This is particularly relevant in *Gambusia* populations, as they show tremendous variation in sex ratios, ranging from 0.1–44.5 (M/F) (Fryxell et al., 2015). Across populations of *Gambusia*, we show that incorporating sex-specific allometric scaling exponents consistently reduces estimates of population-level energy demand (Fig. 3). Males had lower scaling exponents when compared to females ($b = 0.595$ and 0.78 respectively), leading to reduced energy demand in male-biased populations. In our data, the combined male and female slopes were similar to each other, likely due to the larger body sizes in females leveraging the regression line. Nonetheless, this combined regression line does not represent a uniform population with a single underlying scaling slope but instead, a misapplication of regression forced through two groups with different size ranges and slopes. As such, the combined regression misses that males, in particular, may have significantly lower scaling, which could lead to systematic errors in population-level energy demand predictions. For example, in our population with the highest male-to-female ratio, we estimated a 28% reduction in population-level metabolism when using sex-specific scaling compared to scaling exponents where data from both sexes are combined. Such differences in sex ratio among populations and allometric scaling exponents between sexes could lead to substantial ecological changes in ecosystems. For example, Fryxell et al. (2015) found that female-biased *Gambusia* populations had induced stronger pelagic trophic cascades due to increased consumption rates of crustacean zooplankton. This of course, presumes that differences in metabolic demand inferred from field respirometry of populations, largely measuring resting metabolism, translate into metabolic differences in free-ranging fish. In *Gambusia*, male energy budgets likely entail a significant active component associated with mating activity, so that outcome is not assured. Nonetheless, the large difference in population energy demands between sex-specific and sex-neutral models suggests that understanding sex differences could be important to understanding cascading ecological outcomes of sex ratio variation.

Conclusion

Our research demonstrates that reproduction may significantly alter allometric scaling and population-level energy demand. In females, reproduction costs include the fish's pre-pregnancy metabolic rate and increases in metabolism to cover embryo respiration, nutrient provisioning, and other maintenance costs. We show that such costs increase the steepness of the allometric slope of metabolism in females, leading to a disproportionate increase in energetic demand with body size for pregnant individuals. While studies have noted that viviparous species are likely to have increased energetic costs associated with embryo development (Weldon et al. 2013; Ladds et al. 2017; Lima-Santos et al. 2021), we know of no studies that have assessed changes in allometric scaling between reproductively active and inactive stages in a viviparous ectothermic species.

Further, our data suggest how sex differences in allometric scaling could change population-level energy demand. Across populations and species, many factors (*e.g.,* temperature, competition, predation) influence body-size distributions and sex ratios. As such, determining the effects of reproduction and sex on scaling is essential to estimate population energy demand accurately. Overall, our data suggest that differences in energy investment in reproduction between sexes driven by female pregnancy may alter scaling, potentially affecting population energy demand, particularly where sex ratios vary across populations.

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Supplementary Data

Supplementary Data available at *ICB* online.

Data availability

Data are available at DOI: 10.5061/dryad.vg89038 and DOI: 10.5061/dryad.2280gb5ts.

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