

The effect of gestational state on oxygen consumption and response to hypoxia in the sailfin molly, *Poecilia latipinna*

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Synopsis

Metabolic activity in livebearing fishes increases with embryonic development so that embryos prior to parturition may have a higher mass-specific oxygen requirement than maternal tissues, temporarily increasing the total routine oxygen requirement of the female. We examined whether females of the livebearing poeciliid *Poecilia latipinna* (sailfin molly) increase their routine metabolic oxygen consumption during development of their broods. We also quantified effects of gestation on time allocation to aquatic surface respiration (ASR) under hypoxic conditions. Mass-adjusted routine metabolic rate (RMR) of female mollies showed a significant increase during late gestation. The RMR of males did not differ from females that were in their early or mid stage of gestation, but was lower than females in late gestation. Gestating females spent approximately 27% more time conducting ASR than non-gestating females when exposed to chronic hypoxia (1 mg l⁻¹), further supporting a brood-related increase in oxygen demand. Increased time allocation to ASR may directly affect maternal predation risk in low-oxygen conditions.

Introduction

Although viviparity has evolved many times and occurs in almost all classes of vertebrates except birds, less than 3% of teleost fishes are viviparous (Wourms 1981, Meyer & Lydeard 1993). As a form of parental care, livebearing has many advantages to offspring survival. It may offer the embryo protection from predation and environmental extremes in temperature, oxygen, and salinity (Wourms & Lombardi 1992, Goodwin et al. 2002). It may also allow for embryo dispersal into better habitats (Constantz 1989) and the possibility of post-zygotic nutrient provisioning (matrotrophy; Blackburn 1992, Marsh-Matthews et al. 2001). However, there are also costs of livebearing to the female including: The formation of placental analogs and/or uterine nutrient secretions, increased work for the cardiac system, increased branchial ventilation rate, increased cost of

osmoregulation (due to increases in ventilation rate), and increased predation risk due to reduced mobility (Thibault & Schultz 1978, Blackburn et al. 1985, Goodwin et al. 2002).

During gestation, the maternal system must provide oxygen to and remove metabolic wastes from the embryo and must respond to changes in metabolic oxygen requirements of the developing brood (Thibault & Schultz 1978, Blackburn et al. 1985). Early in development of livebearing fishes, most of the embryonic material (water and yolk) is inert and does not respire. However, late in gestation, the actively developing tissues of the embryo may have a higher mass-specific oxygen requirement than maternal tissues and may temporarily increase the mass-specific routine oxygen requirement of a livebearing female (Boehlert & Yoklavich 1984, Boehlert et al. 1986, Dygert & Gunderson 1991).

The following equation summarizes the metabolic oxygen requirements (VO_2) of a gestating female (DeMarco 1993):

$$VO_2 \text{ total} = VO_2 \text{ nonreproductive} + VO_2 \text{ litter maintenance} \\ + VO_2 \text{ embryos}$$

Unfortunately, it is extremely difficult to measure the separate components contributing to the total metabolic oxygen requirement of a female during gestation (DeMarco 1993). Most estimates of the metabolic rate of developing embryos in fishes have been done on either the embryos of oviparous species (DeSilva & Tytler 1973, Houde & Scheckter 1983, Morioka 1985, Oikawa & Itazawa 1993, DeSilva et al. 1986, Walsh et al. 1989, Oikawa et al. 1991) or using *in vitro* techniques on livebearing species (Webb & Brett 1972, Boehlert & Yoklavich 1984, Boehlert et al. 1986, Dygert & Gunderson, 1991).

In the viviparous, primarily marine, genus *Sebastes*, mass-specific oxygen consumption of embryos *in vitro* increases with developmental stage either linearly (*Sebastes melanops*, Boehlert & Yoklavich 1984) or exponentially (*Sebastes schlegeli*, Boehlert et al. 1986, *Sebastes caurinus*, Dygert & Gunderson 1991). Such *in vitro* experiments suggest an increase in oxygen consumption of embryos with development. However, these studies have been criticized because the ambient oxygen levels used during the measurements were higher than generally found in intraovarian fluids, and the embryos may be more active under the stressful *in vitro* procedures (Boehlert et al. 1991).

Comparative measurements of the respiration rate of gestating and non-gestating fish provide a second method for examining changes in mass-specific metabolic rate with development. The significantly elevated respiration rates of gestating female *S. schlegeli* were indicative of an increase in oxygen consumption by embryos as well as an increase in oxygen consumption by the maternal system (Boehlert et al. 1991). The same pattern has been found in other livebearing ectotherms such as the ovoviviparous lizard, *Sceloporus jarrovi* (Guillette 1982, DeMarco 1993).

An increase in metabolic demand of a brood during development would not only be an added energetic cost, but may also increase the risk of predation to a gestating female in hypoxic conditions by significantly reducing metabolic scope of activity and potentially altering respiratory behavior. Under extreme hypoxia, many non-air-breathing fishes use aquatic surface respiration (ASR; pumping the oxygen-rich microfilm at

the surface of water through gills) to increase oxygen uptake (Kramer & Mehegan 1981, Kramer & McClure 1982). This behavior incurs a significant energetic cost as well as an increased risk of aerial predation (Kramer 1983, Kramer et al. 1983). The increased metabolic demand of a brood during gestation may result in a higher behavioral allocation to ASR.

The sailfin molly (*Poecilia latipinna*) is a small, live-bearing, euryhaline species whose range extends from South Carolina southward and westward, throughout Florida and along the Gulf Coast into Mexico (Lee et al. 1980). The species occupies a wide range of habitats that includes both well-oxygenated waters such as flowing rivers and hypoxic waters such as shallow wetlands and stagnant pools (Timmerman 2001). *P. latipinna* is a species that readily breeds in captivity and has a short gestation period (approximately 6 weeks, at 28°C). The sailfin molly does not have specialized structures devoted to matrotrophy; however, Trexler (1985, 1997) provides evidence for facultative matrotrophy in this species. Gestation occurs within the follicles of the ovary (Wourms, 1981), and female mollies must be capable of meeting the metabolic requirements of a brood throughout development even under extreme conditions such as hypoxia. However, whether oxygen requirements of a gravid female vary with development of a gestating brood is unknown. In addition, the effect of brood development on time allocation to ASR under hypoxic conditions has not been previously studied.

This study had two major objectives. The first was to determine whether *P. latipinna* females exhibit an increase in routine metabolic oxygen consumption during the development of their broods. The second objective was to determine whether pregnant females spend a higher proportion of time at the surface (ASR) in response to hypoxia than non-gestating females.

Methods

Metabolic rate experiment

We collected *P. latipinna* from the coastal salt marsh in Cedar Key, Florida. In this habitat, dissolved oxygen concentration is highly variable, both spatially and temporally; and hypoxia (<2.0 mg l⁻¹) is common (Timmerman 2001).

Three aquaria, containing three females and one male each, were maintained with a 12-h photoperiod

at 10 ppt salinity for a period of 14 weeks to capture two reproductive cycles from the females. To minimize duration of reproductive cycles, the fish were acclimated to a relatively high temperature, but one that they experience in the field in the summer (28°C, Timmerman 2001). Fish were tattooed for individual identification (McKinsey & Chapman 1998, Chapman et al. 1999) and acclimated to their aquaria for 2 weeks prior to taking metabolic readings. Water quality was maintained using a sponge filter, and fish were fed Tetra Min[®] flake food *ad libitum* once daily throughout the experiment. A closed respirometry system was used to measure oxygen consumption of individual females weekly and males bi-weekly. Individuals were acclimated overnight in 0.5-l bottles rendered opaque with duct tape. The opacity of the bottle was designed to minimize disturbance to the fish during the sampling period, thereby obtaining a resting routine metabolic rate (RMR, Winberg 1956, Fry 1957). Food was withheld 24 h prior to the weekly RMR reading for each fish. The oxygen level was measured at 5-min intervals by a YSI SONDE water quality probe model 600 immersed in the bottle, and the data were logged electronically using the data collection program, PC6000. To prevent low-oxygen stress to the fish, each reading was stopped, and an aerator placed in the bottle once dissolved oxygen level reached 3.0 mg l⁻¹. Water volume in the bottle was measured, the fish was weighed, its standard length (SL) was measured and then it was returned to its aquarium. Each female's stage of gestation was estimated by back-dating from the date of parturition. In this experiment, one female did not reproduce and died, apparently of senescence, after 7 weeks. The period of gestation was divided into three categories: early (weeks 1 and 2), middle (weeks 3 and 4), and late (weeks 5 and 6). The average of the measurements for each stage from two complete reproductive cycles for each female was used in the analyses. The average of metabolic readings taken across the study period was used for determining the males' RMRs. Mass-adjusted RMRs were used for comparison of gestation period and gender and were calculated following Ultsch et al. (1978) and Ultsch (1995) with the exponent for poeciliids (0.73 = b) proposed by Winberg (1961):

$$\text{VO}_2(\text{adjusted}) = (\text{mean mass of all fish used})^{b-1} \\ \times (\text{observed mass})^{1-b} \\ \times (\text{observed VO}_2)$$

where all VO₂ values are expressed as mg O₂ g⁻¹ h⁻¹.

We used a repeated measures analysis of variance to determine the effect of a female's gestational stage on her RMR. We predicted an increase in RMR with gestational stage, and tank was included as a between-subjects factor in the model. Following Potvin et al. (1990), Mauchly's criterion was used to test for the compound symmetry of the variance-covariance matrix. Repeated contrasts were used to test for differences among the gestational stages. The repeated contrast compares the mean of each stage to that of the subsequent stage. A t-test was used to compare the mean mass-adjusted RMR of males to females at each gestational stage category. We predicted that males would have a lower metabolic rate than females that were in the late stage of gestation. We used one-tailed tests to examine these predictions.

ASR experiment

Forty female sailfin mollies, collected from Cedar Key in August 1998, were marked with tattoo ink for individual identification and acclimated to laboratory conditions (25°C, 10 ppt) for 6 days. This facilitated quick identification of individual fishes during trials. Two 250 l aquaria were divided in half to facilitate easier tracking of individual fish. An attempt was made to balance the number of gravid and non-gravid females in each chamber. Females preliminarily categorized as 'gravid' or 'non-gravid' based on external visual inspection before being placed into each chamber of the two aquaria. For each aquarium, there was a total of 20 fish, five 'gravid', five 'non-gravid' fish on each side. At the conclusion of an experiment, each female was euthanized with MS222, weighed, and measured. Each female was then dissected to determine the stage of her brood (Haynes 1995).

Dissolved oxygen levels were steadily reduced using a microprocessor-based oxygen controller that bubbled nitrogen or air slowly into the aquaria until the specified dissolved oxygen level was reached. Fish were held at 1.0 mg l⁻¹ O₂ overnight prior to taking behavioral readings. We used 1.0 mg l⁻¹ O₂ (at 25°C), because we had preliminary data to show that this level induces ASR behavior in mollies. Behavioral data consisted of noting the individuals conducting ASR every 10 s over a period of 3 min. This was repeated 10 times for each chamber over 2 days. The average percent of time an individual was seen at the surface was calculated. Fish were classified as non-reproductive, or reproductive if they were in any stage of gestation. Stages of gestation

were not compared because early stages were not well represented in the sample.

Linear regression was used to test for an effect of body size on percent time using ASR (% ASR). To test for an aquarium effect, the mean % ASR for reproductively active and reproductively inactive fish were separately compared between the two aquaria. Because no significant aquarium effects were detected, we combined females across the two aquaria to test for a difference in mean % ASR between reproductively active and reproductively inactive individuals.

Results

Metabolic rate

Repeated measures ANOVA indicated no effect of tank on the RMR of female mollies ($F = 0.052$, $p = 0.950$). However, there was a significant effect of reproductive stage on mass-adjusted RMR both when tank was included as a between-subjects factor ($F = 4.789$, $p = 0.018$) or when tank was removed from the model ($F = 5.285$, $p = 0.010$, Figure 1). There was no difference in mass-corrected RMR (adjusted to a mean body mass of 2.06 g) between early ($0.333 \pm 0.037 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1}$) and mid-gestation ($0.334 \pm 0.031 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1}$); however, there was a significant increase during late gestation ($0.424 \pm 0.037 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1}$, repeated contrasts, $p < 0.05$, Figure 1). Because we detected no tank effects,

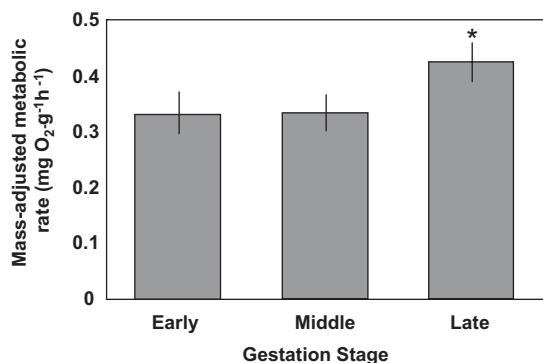


Figure 1. The mass-adjusted metabolic rate ($\text{mg O}_2 \text{ g}^{-1} \text{ h}^{-1} \pm \text{SE}$) of female *P. latipinna* at the early, middle, and late stage of gestation (least squares means). Fish were collected from Cedar Key, Florida. Metabolic rates were adjusted to 2.06 g body mass following Ultsch et al. (1978, 1995). * indicates that the metabolic rate at the late stage of gestation was significantly higher than the previous stage (repeated contrasts, $p < 0.05$).

we compared the RMR of males to females that were combined across tanks. Mass-adjusted RMR of male mollies ($0.292 \pm 0.033 \text{ mg O}_2 \text{ g}^{-1} \text{ h}^{-1}$) did not differ from females in the early stage ($t = 0.643$, $p = 0.268$) and mid stage ($t = 0.787$, $p = 0.226$) of gestation. However, mean mass-adjusted RMR of males differed from females measured during the late stage of gestation ($t = 2.106$, $p = 0.032$).

ASR experiment

Linear regression analyses indicated no relationship between body mass and % ASR for females with gestating embryos ($r^2 = 0.040$, $F = 0.755$, $p = 0.396$) or females without embryos ($r^2 = 0.118$, $F = 2.283$, $p = 0.149$). Therefore, body mass was not used as a covariate in comparing % ASR between groups. There was no difference in the mean % ASR of non-gestating individuals between aquaria one and two (mean for aquarium one = 16.82 ± 3.01 , SE; aquarium two = 12.15 ± 3.76 , $t = 0.978$, $p = 0.342$). Similarly, there was no difference in the mean % ASR of gestating females between aquaria one and two (mean for aquarium one = 43.75 ± 7.58 , SE; aquarium two = 40.7 ± 8.04 , $t = 0.257$, $p = 0.799$). Therefore, we combined females from both aquaria to test for a difference in mean % ASR between gestating and non-gestating females. Percent ASR was much higher in gestating females than in non-gestating females ($t = 4.48$, $p < 0.001$, Figure 2).

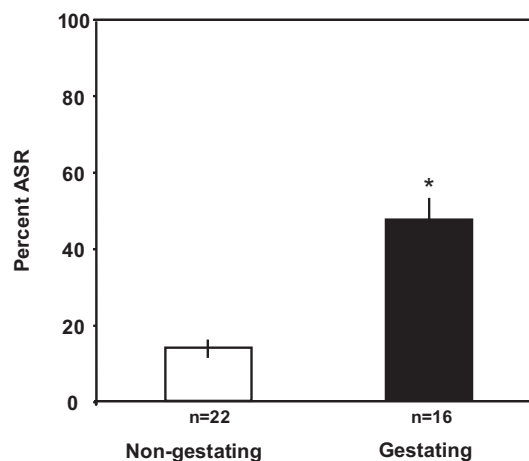


Figure 2. A comparison of mean % ASR ($\pm \text{SE}$) for non-gestating and gestating female mollies *P. latipinna* collected from Cedar Key, Florida. * $p < 0.05$. Fish were exposed to a dissolved oxygen level of 1 mg l^{-1} .

Discussion

The results of this study agree with earlier work on other livebearing species (*S. melanops*, Boehlert & Yoklavich 1984; *S. schlegeli*, Boehlert et al. 1986; *S. caurinus*, Dygert & Gunderson 1991). For example, the livebearing scorpaenid fish, *S. schlegeli* increases its RMR by as much as 68% just prior to parturition (Boehlert et al. 1991); while the livebearing surfperches (*Rhacochilus vacca* and *Embiotoca lateralis*) increase their metabolic rate by 53% during late gestation (Webb & Brett 1972). The isometric relationship between mass and metabolic requirements determined for several larval fish species may be associated with the higher proportional mass of metabolically demanding tissues (the brain and kidneys) relative to less energetically active tissues (trunk and white muscle) (Oikawa et al. 1991). When almost half of a female sailfin molly's body mass constitutes her developing brood, and she may carry as many as 80 embryos at one time (pers. observ.), the additive effect of the brood's metabolic cost of organogenesis could increase total maternal metabolic oxygen requirement. In this study, the stage of gestation appears to be an important factor in determining the metabolic oxygen requirements of female *P. latipinna*, leading to a significant increase in mass-specific oxygen consumption during the final third of gestation.

It has been suggested that the increased embryonic metabolic oxygen requirements with development reduce dissolved oxygen levels within the lumen of the follicle, eventually stimulating an embryonic stress response as a normal component of parturition initiation in *P. latipinna* (Guillette 1987). Measurements of intraovarian oxygen tension in livebearing surfperches (*R. vacca* and *E. lateralis*) have shown a decrease in tension with increasing metabolic demands of the brood, reaching a minimum of 13.7 mm Hg just prior to parturition (Webb & Brett 1972). Anecdotal evidence from metabolic critical tension experiments on sailfin mollies supports the idea of a possible link between embryonic hypoxia stress response and parturition (Timmerman 2001). Exposure to extreme hypoxia led to premature parturition of late-stage embryos on several occasions. In contrast, females with unfertilized ova or young embryos were not observed to abort under similar conditions. Late in gestation, the follicle may have increased sensitivity to the embryonic release of adrenal corticosteroids and catecholamines, and this stress response may initiate parturition (Guillette 1987).

In response to hypoxia, gestating females spent approximately 27% more time conducting ASR than non-gestating females, further evidence of a brood-related increase in oxygen demand. Differential recruitment of organs throughout ontogeny is considered to be an important factor in the biphasic pattern in metabolism-mass scaling observed for many fish species (Post & Lee 1996). If groups in this ASR experiment could have been partitioned by the embryonic stage of organogenesis or recruitment of organs, the differences in time spent conducting ASR by females in different gestational stages, may have been determined with more resolution.

Poeciliids in general are effective at conducting ASR, having a head morphology that is well adapted to that purpose. Their supra-terminal mouth and dorso-ventrally flattened head allow access to the air-water interface without breaking the surface of the water (Lewis 1970). Several species in the family Poeciliidae have been reported to use ASR: *Poecilia gillii* (Chapman & Chapman 1993); *Gambusia holbrooki* (Cech et al. 1985, McKinsey & Chapman 1998); *Poecilia sphenops* and *Xiphophorus helleri* (Kramer & McClure 1982); and *Poecilia reticulata* (Kramer & Mehegan 1981, Weber & Kramer 1983). Populations faced continuously with low-oxygen conditions often alternate other activities, such as feeding and reproduction, with periodic bouts of ASR (Weber & Kramer 1983, Chapman & Chapman 1993). However, there are high costs to ASR. Under extreme hypoxia, many fish species that use ASR have been observed to spend over 90% of their time at the surface (Gee et al. 1978, Kramer & Mehegan 1981, Kramer & McClure 1982, Chapman & Liem 1995, Olowo & Chapman 1996).

The increased time allocated to ASR that we observed in gestating female mollies may directly affect maternal predation risk in low-oxygen conditions. In some fishes, the risk of aerial predation increases with time spent at the surface conducting ASR (Kramer et al. 1983). Additionally, egrets and herons appear to prefer larger sailfin mollies (Trexler et al. 1994). The larger silhouette of a gravid female at the surface would probably be more visible to aerial predators than the same female in a non-reproductive state. Females near parturition may be more vulnerable to aerial predators, spending more time at the surface at a time when they would already be a preferred prey item of some wading birds. In the field, one might anticipate behaviors by gestating females to reduce the risk such as selection of microhabitats with elevated

levels of dissolved oxygen or areas that reduce aerial predation risk such as vegetated cover. Future studies linking gestational state with field micro-distribution and predation rate data may provide additional evidence for the potential cost of gestation under hypoxia in female livebearing fishes.

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