

Sex differences in oxygen consumption in Japanese medaka (*Oryzias latipes*)

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Abstract

One of the defining characteristics in sexual dimorphism is that there are morphological differences between the sexes. Sexual selection favors traits that are advantageous for reproduction, usually acting on males due to their higher variability in reproductive success, often resulting in sexual dimorphism. In Japanese medaka (*Oryzias latipes*), sexual dimorphism can be observed in the larger size of male anal fins, which functions in reproduction, and larger body size in females which results in increased fecundity. Large body size in males is attributed to greater mating success compared to smaller body size males. To assess whether or not dimorphism is related to physiological differences between males and females, I examined the rates of oxygen consumption between the sexes to determine whether higher weight contributed to differing oxygen consumption. Individual medaka were assessed for oxygen consumption over a 30-minute time period. Weight was moderately positively correlated with increasing oxygen consumption in males, and there was no correlation for females. Males had a higher average weight than females (0.168g vs. 0.159g). There were differing rates of oxygen consumption between the sexes not influenced by weight during the trials, suggesting that there may be additional physiological differences which are responsible for the difference in oxygen consumption between the sexes.

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Introduction

Sexual selection is often defined as advantages that individuals of a sex have over other members of that sex, specifically in terms of reproduction (Hosken & House 2011). This idea was coined by Charles Darwin as a definition to explain why specific traits, which appear to have a negative impact on survival, may evolve and persist within species (Darwin 1872). These traits can vary from ornaments used to exploit sensory bias such as orange color in guppies being preferred by females (Andersson & Simmons 2006; Rodd et al. 2002) to characteristics that give the animal increased rates of fertilization such as large body size in male mosquitofish (*Gambusia holbrooki*) (McPeck 1992). This occurs because alleles that are desired in a population will be passed on to offspring, who in turn will have more success finding a mate (Mays & Hill 2004).

Sexual selection has two main mechanisms: intersexual choice and intrasexual competition (Parker 1979). Intrasexual competition is defined as competition within a sex for access to resources or mates (Bateman 1948). Intrasexual competition will often give advantages through differing morphologies in males such as increased size in defensive and offensive armaments (Slatkin 1984). These armaments can provide offspring increased survival function or increased mating success (Slatkin 1984). In mating systems with choice components, intrasexual competition facilitates intersexual choice, which is defined as one sex choosing a mate based on desirable traits to assist in increasing their offspring's survival or reproductive success (Moore 1990). Intersexual choice is predominantly female-choice due to the additional energy requirements of producing eggs compared to that of

producing sperm (Andersson & Iwasa 1996). These additional energy requirements can be observed through the production of gamete biomass, where females can produce up to four orders greater than males (Hayward & Gillooly 2011). Intersexual choice adds selective pressure on mating systems, as reproduction will primarily occur with males that the females prefer (Parker 1979). These mechanisms select for observable differences between the sexes, creating sexual dimorphism (Slatkin 1984).

Sexual dimorphism can be defined as morphological differences between the sexes within an animal species (Slatkin 1984). Sexual selection acts on male traits, resulting in sexual dimorphism (Slatkin 1984). Darwin (1872) believed that sexual selection alone is not the sole reason for sexual dimorphism, and that dimorphism must evolve in other ways (Hedrick & Temeles 1989). One of these ways is the reproductive role hypothesis, which suggests the most important reproductive role for females is to ensure their energetic needs are met to ensure egg production (Bulté et al. 2008). Sexual niche overlap is defined as members of opposing sexes competing for the same resources within a habitat (Hedrick & Temeles 1989; Selander 1972). If sexual niche overlap is present through food competition, traits can evolve to increase the size or efficiency in feeding structures, causing dimorphism (Bulté et al. 2008). Through sexual dimorphism, the sexes are able to occupy separate niches increasing their chances of survival, and ensuring females' energetic needs are met (Selander 1972).

Females are typically larger than males because fecundity increases with increasing weight (Hirshfield 1980). However, in aquatic animals, the sizes of the sexes are often very

similar (Howard et al. 1998; Parker 1992). This is thought to occur because of increased mortality with size outweighing the benefits of increased fecundity (Howard et al. 1998). Additionally, relatively large size in males is often attributed to increased mating success as females will receive superior genes and higher sperm quality (Williams 1975). However, large size in males and females may result in more oxygen consumed through locomotion in water unless there are secondary sexual characteristics that offset those differences such as anal fins (Brett 1965).

In aquatic animals, energetic behaviors can have an additional cost associated with them due to the medium in which they live (Hind & Gurney 1997). As water is thicker than air, this results in more resistance on aquatic animals than terrestrial animals (Beal 1946). This resistance is defined as drag, which is the friction that is created when an organism moves through a medium (Alexander 1992). Fish mitigate drag through the presence of anal fins, which allow the fish to become more streamlined (Han et al. 2020). In bluegill sunfish (*Lepomis macrochirus*) larger anal fins allow for a larger displacement of water, reducing the drag while moving through water (Han et al. 2020).

The Japanese medaka (*Oryzias latipes*) is a lowland killifish that lives in ponds, rice paddies, and other similar wetland locations (Roberts 1998). Sexual dimorphism in medaka can be observed through relatively large anal and dorsal fin size in males, increasing the proportion of fertilized eggs, as the males can envelop the female and bring his urogenital zone closer to hers for more efficient fertilization (Koseki et al. 2000). It may also be observed through body size and weight as females tend to be heavier and larger than males

(Hirshfield 1980). Larger medaka have relatively high fecundity, suggesting that there is sexual selection on this trait (Howard et al. 1998). Additionally, due to the increased proportion of fertilized eggs with longer anal and dorsal fins, females prefer males with larger anal and dorsal fins, suggesting that there is sexual selection on this trait as well (Fujimoto et al. 2014).

The primary goal of this research is to determine whether weight and oxygen consumption are positively correlated. I predict that there will be a positive correlation between weight and oxygen consumption (Brett 1965; Han et al. 2020). A secondary objective of my research is to determine whether males or females have higher base rates of oxygen consumption per milligram of weight. I predict that females will consume more oxygen than males, as they are typically heavier than males (Brett 1965; Han et al. 2020; Howard et al. 1998).

Methods

2.1 Experimental Animals

Ten male and ten female mature Japanese medaka (*Oryzias latipes*) were selected at random from Saint Mary's University's Aquatic Facilities in Halifax, Nova Scotia. Fish were weighed using a TE153s analytical balance to the nearest 0.001g, and their lengths measured using Whitworth 6' 150mm digital calipers to the nearest 0.01mm. Each fish was placed into an individual Tupperware container to be held one hour prior to the experiment.

2.2 Experimental Design

The metabolic rate of males and females were measured at rest to assess the initial differences between males and females. The average length and weight of males was 20.74mm and 0.168g, and the average length and weight of females was 20.89mm and 0.159g.

2.3 Experiment

2.3.1 Chamber Setup

Prior to the measurements, a closed system respirometer was set up to measure oxygen consumption of the medaka. It consisted of a 109mL glass container, equipped with a small stir bar at the bottom covered with a piece of mesh secured to the container to avoid contact with the fish. A 2mm Loligo oxygen sensor spot was adhered to the inside of the glass container. A fibreoptic cable was seated into a hole bored into a standard eraser that was secured to the outside of the container. Oxygen saturation (mgO_2/L) of the water was measured using a Witrox 4 reader connected to a computer via Bluetooth, and readings

were monitored using AutoResp v2.3.0, a software which measures oxygen consumption in aquatic organisms.

2.3.2 Calibration

The respirometer was calibrated for 0% and 100% oxygen saturation between trials. To zero for lack of oxygen, 0% was calculated by filling up the container to the top with 0.32M Na₂SO₃, and the lid was screwed on. The magnetic stirrer was turned on to circulate the Na₂SO₃. The respirometer was left until the oxygen saturation remained unchanged for 5 minutes, and then its value was locked as the 0% marker. The chamber was rinsed out three times using 95% EtOH, and then was rinsed out three times using reverse osmosis deionized (RODI) water.

Once the container was rinsed thoroughly, the magnetic stirrer was turned off, the container filled partially with tank water, and an air stone connected to a Frabill Aqua Life aeration system was inserted into the water to bubble the water. The fibreoptic cable was seated into the eraser, and once readings remained unchanged for 5 minutes, 100% oxygen saturation was locked. These steps were performed with every trial.

2.3.3 Data Collection

The container was filled with water from an aquarium, and a single fish was placed inside. The container was sealed and the magnetic stirrer was turned on to circulate the water. The volume of the container inserted and the weight of the specimen were inserted as parameters as the experiment was started on AutoResp. Once the experiment started, Close Respirometry was selected. Once the trial began, the lights were turned off. Oxygen

saturation of the container was measured for half an hour, and this was repeated for each of the 20 medaka.

2.4 Statistical Analysis

Generalized linear models was used to show the percent difference of oxygen saturation after 30 minutes compared between males and females. An ANCOVA test was used to analyze the correlation of increasing weight to differences in oxygen saturation. A Welch two sample t-test using male and female residuals was used to analyze the comparison of metabolic rate. All analyses were done using R v4.1.1.

Results

3.1 Oxygen Saturation and Fish Weight

Specimen weight and percent difference in oxygen saturation (mg O₂/L) was measured to quantify the relationship between the variables. Specimen weight was measured to the nearest thousandths of a gram using a tared container of water on an analytical balance. Percent difference in oxygen saturation was measured through monitoring the change of oxygen saturation in the chamber and collecting the difference in oxygen saturation over 30 minutes.

For the 20 trials comparing difference in oxygen saturation to weight, an ANCOVA test was done linking the interaction of sex and percent difference of oxygen saturation ($F_{1,16} = 5.644$, $p = 0.030$). A one-way analysis of variance showed that there is a positive relationship between oxygen consumption and weight ($F_{1,8} = 7.748$, $p = 0.024$; Figure 1). Oxygen consumption did not increase with weight for females ($F_{1,8} = 0.186$, $p = 0.68$; Figure 1).

3.2 Oxygen Consumption Analysis

I compared the metabolic rates of males and females under identical conditions in order to determine the differences between the sexes. This was quantified by monitoring the change of oxygen saturation (mg O₂/L) in the chamber and collecting the difference in oxygen saturation over 30 minutes. These values were divided by the weight of the specimen (mg) and by the amount of time spent (min). Males' metabolic rate (mg O₂/L/mg

body weight/min) (mean \pm standard error (SE) = $5.586 \times 10^{-4} \pm 1.212 \times 10^{-4}$) and females' ($8.358 \times 10^{-4} \pm 2.094 \times 10^{-4}$) were not significantly different ($t = 1.1456$, $p = 0.27$; Figure 2).

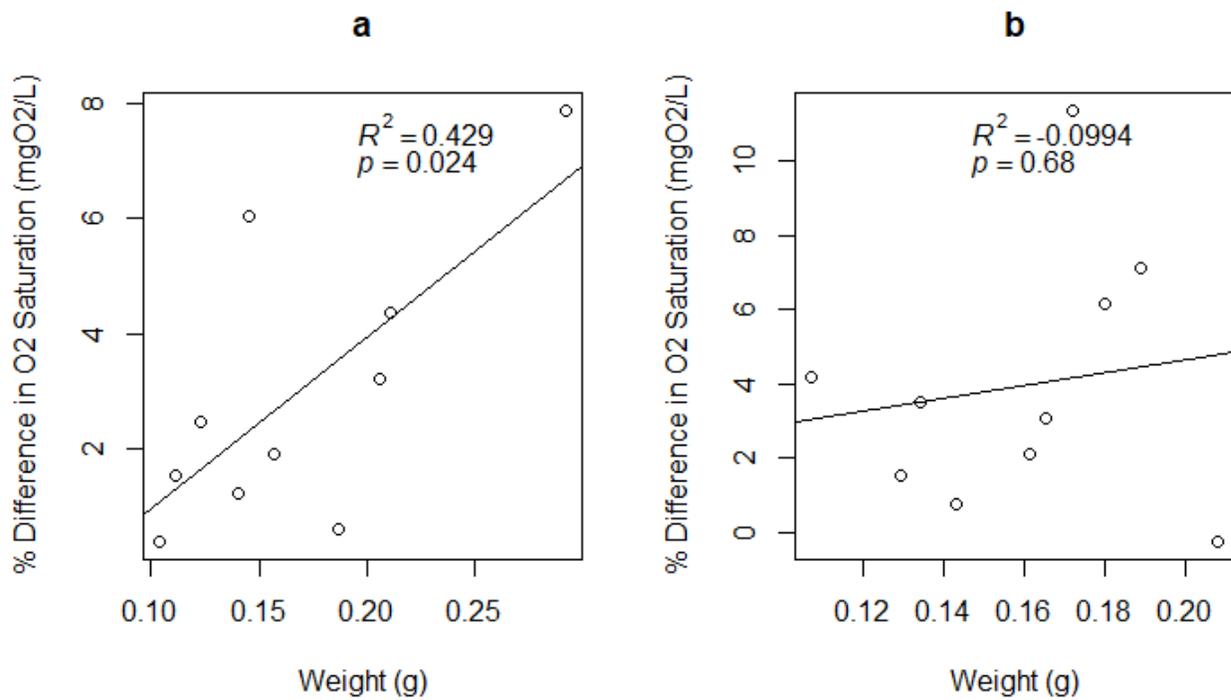


Figure 1. Percent difference in oxygen saturation (mg O₂/L) in male (a) and female (b)

Oryzias latipes over 30 minutes compared to weight (g), n = 10.

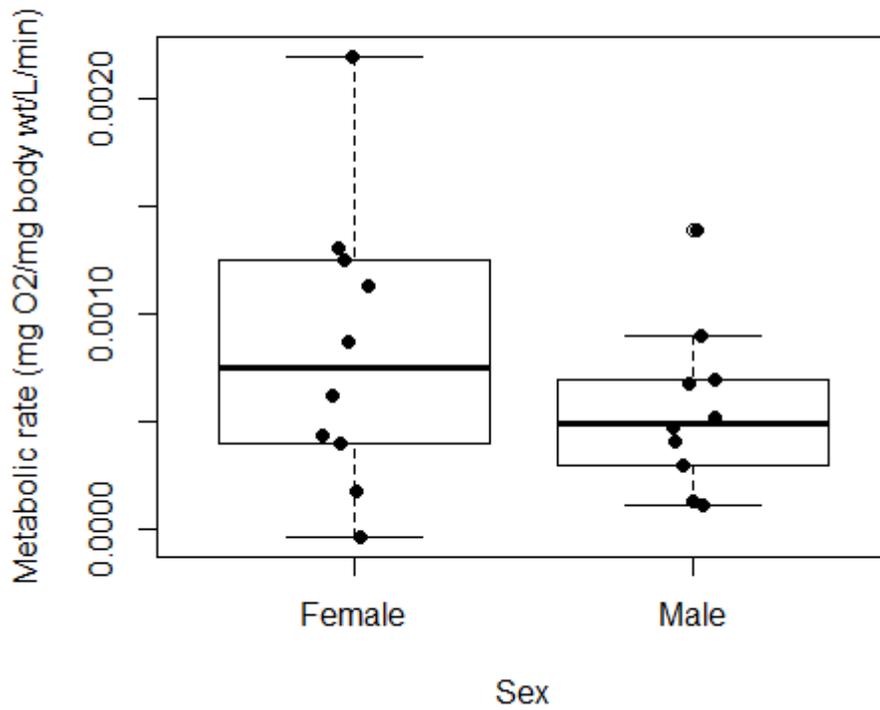


Figure 2. Boxplot of metabolic rate (mg O₂/mg body weight/L/min) in female *Oryzias latipes* compared to males. Black line represents the median, and circles represent individual medaka, n = 10.

Discussion

The purpose of this study was to determine whether there was a positive relationship between weight and oxygen consumption in medaka, and to compare those values between the sexes to identify differences in oxygen consumption between the sexes. I first predicted that weight positively correlated with oxygen consumption due to a higher muscular demand of oxygen to continue locomotion in heavier animals (Brett 1965). In partial support of my prediction, I found a positive relationship between male weight and male oxygen consumption; however, I found that female weight and female oxygen consumption were uncorrelated.

This lack of correlation may have occurred due to not allowing the fish to acclimate in the respirometry chamber, or a female reading at a negative percent difference in oxygen saturation. It is possible that the fiberoptic cable was improperly seated in the eraser, causing that anomaly. A potential improvement could be to create a sturdier part to hold the fiberoptic cable to ensure that it locks in place, to measure multiple times with the same fish to ensure accuracy, and to give the fish time to acclimate in the chamber prior to measurements. Additionally, finding methods to observe or control for fish locomotion could allow more precise methods for measuring for resting metabolic rate.

My second prediction was that females would have a higher rate of oxygen consumption than males as the production of ovum would increase the rate of respiration compared to males (Boehlert et al. 1991). For my second prediction, I found that males did not consume less oxygen than females during the study.

These findings may have occurred due to the male medaka being heavier than the females on average. Additionally, there was a female who read at a negative percent difference in oxygen consumption. A potential improvement could be to add additional measurements with the same specimen; if the steps were repeated with multiple trials, it could provide more accurate data.

4.1 Increasing Weight and Oxygen Consumption

Male oxygen consumption was positively correlated with weight. However, female oxygen consumption was not influenced by higher weight. These findings are partially consistent with Brett (1965), who found that higher weight resulted in higher quantities of oxygen that was required for a heavier animal to move through water.

However, these findings are not consistent with Boehlert et al. (1991), whose findings indicated greater respiration rate in female *Sebastes schlegeli* has a positive correlation with weight. To account for these findings, there may be physiological differences in *Oryzias latipes* that may explain these findings in more detail. The liver of female *Oryzias latipes* is shown to differ from males as their relatively greater activity of protein synthesis results in higher energy requirements of female hepatocytes (Qiao et al. 2016). As larger females have greater quantities of hepatocytes, studies have shown greater quantities to reduce the metabolic cellular activities of individual cells; that may explain the lack of an increase in oxygen consumption with size (Porter & Brand 1995). It is likely that more rigorous protocols and increased measurements and observations would result in explanations of these findings.

4.2 Intersexual Differences in Oxygen Consumption

Females did not consume more oxygen than males. These findings are not consistent with Boehlert et al. (1991), who found that due to the production of oocytes in females, their oxygen requirements are higher than males. Due to constant egg production in female *Oryzias latipes*, their oxygen requirements should be typically higher than males (Boulekbache 1981; Leaf et al. 2011). These findings may be explained through the methods taken to collect data: as the specimens' percent difference in oxygen consumption was measured once, it is possible that more collections could provide a more accurate representation of the individual's oxygen consumption. Moreover, the specimens were not observed during the trial in order to limit distractions. If the specimens were observed remotely, that could potentially explain some anomalies in the dataset.

As female *Oryzias latipes* were not larger than males in this study, it may also explain why their oxygen consumption was not significantly higher than males'. Females were lighter on average due to one male being several deviations heavier than the average (0.292g vs. mean = 0.168g). That male was shown not to be an outlier once standard residuals were collected, ($e = 2.8$) but it explains why females were lighter in this study.

Additionally, larger eggs generally require more oxygen to be produced (Rombough 2007). If that is true, it is possible that small females would require a greater amount of oxygen to produce the same size egg as larger females, as they would be more invested in egg production to keep up (Iwamatsu 1994; Hirshfield 1980).

4.3 Conclusion

This research may be used to help in establishing an experimental approach for measuring metabolic rate in *Oryzias latipes* in order to answer questions about their metabolism and metabolic rate.

Additionally, this may be used to gain a stronger understanding of the cost of different activities in *Oryzias latipes*, now that resting oxygen consumption has been acquired. Learning more about the metabolic costs of mating rituals in *Oryzias latipes* may allow researchers to apply this knowledge to other fish species, to explore metabolic costs of mating rituals in greater detail in both the medaka and other aquatic animals.

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