# INFLUENCE OF OPERATIONAL SEX RATIO, SIZE, AND BODY COLORATION OF MALES ON MATE SELECTION AND MALE-MALE COMPETITION IN *Poecilia reticulata* (GUPPIES)

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The operational sex ratio (OSR) in fishes fundamentally describes the intensity of sexual selection and potential reproductive success. It reflects the ratio of sexually active males to sexually receptive females, influencing the intensity of competition, including associate behaviours among males for mates. This research used wild guppies as the model organism to understand the influence of OSR in the behavioural display of both males and females, and to evaluate the relationship between male body colouration, body size of both sexes, and mating success. Methodology consisted of initial collection and acclimatisation of wild guppy populations from the canals around Colombo, like the Udahamulla Railway Station (6.8622720, 79.9086026), Pagiriwaththa Railway Station (6.8648125, 79.9024510), and Paramitha Dharmayathana Temple (6.8763484, 79.9083455), followed by subjecting them into three tests based on OSR, tail colouration of male guppies, and body size of both sexes. Different OSR levels were created, ranging from 1:5 (female-biased) to 5:1 (Male-biased). Under each level, mating tactics such as sigmoid displays (SG), sneak attempts (SA), mirror action (MA), and male-male interactions were recorded using videography tools. Measurement of tail colour and body size preference also consisted of the same behavioural recording protocol. Results indicated prominent SG display while male behaviour declined from 0.17 to 0.83 OSR treatments. The second test of tail colour preference indicated a higher preference for orange-tailed males by the females. In the last test of body size preference, both sexes preferred large-bodied counterparts with increased activity. Hence, the OSR influences the mate choice of both sexes and competition in the more biased sex, which ultimately affects the evolutionary trajectory of the species in the environment through secondary sexual traits.

*Keywords*: operational sex ratio, OSR, guppy, mating behaviour, sexual selection

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## INTRODUCTION

Measuring or predicting the strength of sexual selection in animals poses significant challenges due to the complexity of mating systems (Rueger *et al*., 2024). One recognized method developed to address this is the operational sex ratio (OSR) (Monteiro *et al*., 2013). The OSR significantly influences the reproductive success, which can vary across species, between populations, and even within a single population over multiple generations (Wacker *et al.*, 2013). The general theory of OSR states that when there are more individuals of one sex than the other in a mating population, the sex that is more abundant tends to compete more intensely for access to mates and in contrast, the less abundant sex becomes more selective when choosing a mate (Wacker *et al*., 2013). The sex that reproduces more, quickly shows intense competition for mates, both with respective costs and benefits (Balshine-Earn, 1996).

Guppies are commonly used as model organisms because their adult sex ratio (ASR) varies significantly across different populations and even between pools within the same water body (Jirotkul, 1999). Therefore, in-situ studies can be indicative of the dynamics of wild populations (Jirotkul, 1999).

OSR has broad applications, including identification of the more competitive sex, uncovering mating system patterns and their evolutionary trajectories, and comparing the strength of sexual selection across species, where a strongly male-biased OSR often correlates with prominent male ornamentation (Janicke & Morrow, 2018). Additionally, OSR insights contribute to the long-term management of endangered species by guiding strategies to balance mating dynamics and enhance genetic diversity through informed manipulations of sex ratios (Janicke & Morrow, 2018).

The main objectives of this experimental study are to assess how different operational sex ratio (OSR) conditions affect the behavioural displays exhibited by both males and females, and to evaluate the relationship between male body colouration, body size of both sexes, and mating success under different OSR scenarios.

## METHODOLOGY

Adult breeding-size *Poecilia reticulata* (males: 1.5–2 cm; females: 2.5–2.8 cm) were collected from canals near wetlands (approximately 150 per location) in Colombo and transported to the laboratory in oxygenated polythene bags. Sex was identified using external morphology (Shahjahan et al., 2013). Fish were acclimatised for one week in aerated, aged tap water under natural photoperiods and fed commercial pellets at 5% body weight daily at 08:00 h. After acclimatization, individuals were transferred to separate tanks for behavioural observations. All tanks were partially covered to minimize visual disturbances, and behaviours were recorded via digital camera. Each test was conducted in triplicate using new fish to prevent pseudo-replication.

Three behavioural tests were conducted. To identify the relationships among different levels of OSR and behavioural tactics of male and female *P. reticulata*, OSRs were manipulated while maintaining constant density, using male: female ratios of 1:5(female-biased) to 5:1(male-biased). Male mating tactics—sigmoid displays (SG), sneak attempts (SA), male-male interactions (MM) and mirror action (MA) of females were recorded at 09:00, 12:00, and 15:00 h, along with courtship time, mate-guarding, refractory period, and female responses such as looking at the displaying male (Lm) or following the displaying male (Fm).

Further, female mate preference based on the tail colouration of males was assessed by introducing two males with consistent colour patterns and four females per tank. In addition, the males with different tail colour patterns (e.g., yellow, orange, and white) were mixed and continued the experiment to identify the colour preference of females. Finally, to evaluate the influence of male and female body size on reproductive behaviour, pairs of differently sized males or females were introduced with size-matched counterparts. All observations followed the same behavioural recording protocol. A General Linear Model (GLM) was used to assess the effects of OSR on four different behaviours in male guppies. To analyze the impacts of the body size of males on female and males' behaviours and to test the impacts of tail colours of males on the females' responses, the TWO-Way Permanova was used with 9999 permutations. Man-Witney U test was used to analyse the significance of specific behaviours between large and small-bodied males. Non-linear regression, and where appropriate, linear regression models were used to analyze the relationships among multiple variables.

## RESULTS AND DISCUSSION

Under the test for OSR manipulation for the male fish, sigmoid behaviour of the male was the most frequently observed behaviour type while male-male interactions occurred the least. Overall, male behaviour declined at low (0.17) and high (0.83) OSR treatments. Sigmoid behaviour indicated a negative relationship with OSR ($p=0.032$). Sneak attempt behaviour indicated a significant reduction towards the intermediate OSR which slightly increased with higher OSRs ($p=0.038$**).** A positive relationship was observed with male-male

interaction behaviour and OSR treatments (p=0.482). Overall, orange tailed males were preferred by the females significantly, while white males were rarely chosen by the reproductively active females.

The Lm exhibited the highest female behaviour, with $9.33 \pm 2.11$ occurrences. The Fm behaviour showed 5.4±1.20 occurrences while there was no observation of rm in any test aquaria.

The impact of body size on the reproductive behaviour of both sexes indicated that the behavioural patterns of larger males were usually observed to be higher ($4.83 \pm 1.80$) than smaller males ($2.37 \pm 0.90$), out of which the sigmoid behaviour was prominent. The females responded respectively to the increased behaviour of larger males ($0.916 \pm 0.41$) compared to smaller males ($0.166 \pm 0.11$) by performing a mixed pattern of behaviour. The results of the male preferences for differently sized females indicated that they preferred large-bodied females.

As expected, we observed intensified competition among males for mates due to the relatively lower number of females in higher male-biased OSRs. This imbalance may influence male mating strategies, male reproductive success, and female mate selection. These findings support previous studies on OSR manipulation (Jirotkul, 1999). The implication of this finding, when applied to the wild population, is that as both sexes prefer novel mates and due to increased male-male competition, males move from one location to another to seek better OSRs, which is more female-biased (low OSR) as an adaptive dispersal strategy (Croft et al., 2003). Similar behaviour is also indicated in Milkweed beetles and water striders, suggesting a relatively common scenario (Croft et al., 2003). As there are fewer studies conducted in relation to OSR in guppies or any other fish species globally and locally, this study highlights the necessity of such studies to understand the ecological and evolutionary implications of sex ratio dynamics in fish populations.

## CONCLUSIONS/RECOMMENDATIONS

OSR is a plausible factor that influences trait evolution in guppies in terms of colour, spot appearance, tail morphology, etc. Male reproductive behaviours are highly dependent on the OSRs. Further, reproductive behaviours of both sexes are influenced by the male and female body sizes. In addition, male tail colouration has a significant impact on female behaviours. However, OSR alone cannot influence it as other factors such as predation pressure, genetic constraints, resource availability, and environment can play significant roles in trait evolution (Head *et al*., 2007). These factors need to be further analysed for a thorough understanding of the driving forces of sexual selection in fishes such as guppies.

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