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Metabolism, Cognition, and Male Coloration in Eastern Mosquitofish

by

Katelyn Ashleigh Giltner

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Male Coloration in Eastern Mosquitofish



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Abstract

Since genetic variation is a key factor in adaptation, the mechanisms that maintain this variation are a central topic in evolutionary biology. In particular, life history trade-offs and differences in pace of life between individuals and populations can contribute to maintaining variation in wild populations. *Gambusia holbrooki*, or eastern mosquitofish, display a rare melanistic coloration in a small percentage of males, and these males display traits that may be associated with a faster pace of life. This study examined two potential mechanisms, metabolism and cognitive ability, that may have roles in maintaining the melanistic phenotype in male *Gambusia holbrooki*. Standard metabolic rate was measured with a closed-chamber respirometry approach, and cognitive flexibility was measured using a detour task. Neither standard metabolic rate nor cognitive flexibility varied significantly between silver and melanistic males, which does not support a role for pace of life differences between the two phenotypes. Broader measurements of metabolic scope and cognitive ability may be necessary to illuminate any life history differences between these two color phenotypes that may aid in the persistence of the melanism allele.

Introduction

Genetic variation within populations is crucial to adaptation to a changing environment (Barrett & Schluter 2008). What mechanisms maintain genetic variation in wild populations remains a central question in evolutionary biology. In this study, I examined two potential mechanisms that may aid in the maintenance of a simple genetic polymorphism in male coloration: differences in cognitive abilities and metabolic rates. Specifically, I focused on variation in melanistic and normal coloration in eastern mosquitofish populations.

The eastern mosquitofish (*Gambusia holbrooki*) is a small, live-bearing freshwater fish that is native to the southeastern United States and Mexico. Eastern mosquitofish have been introduced around the world in efforts to control mosquito populations, and they are now invasive on every continent except Antarctica (Froese & Pauly 2010).

Rather than possessing typical silver scales, some male eastern mosquitofish may exhibit a melanistic phenotype with mottled black scales (fig. 1). Melanic males also tend to exhibit increased aggression, sexual activity, and larger body sizes (Horth 2003; Horth et al. 2010).

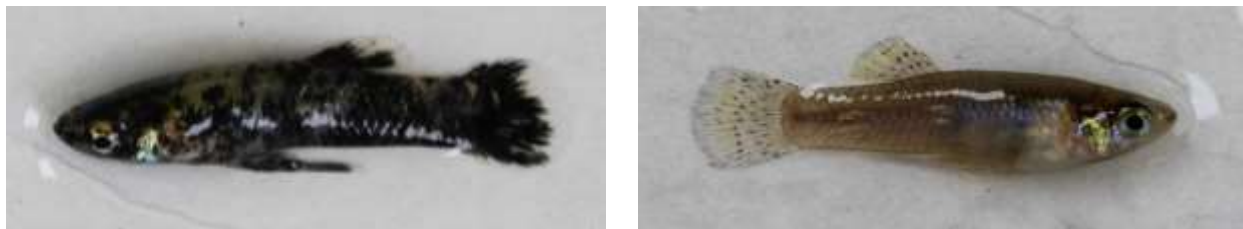


Figure 1 – Melanistic (left) and silver (right) color phenotypes in male *Gambusia holbrooki*.

Life History & Pace of Life

Life history traits are the characteristics of an organism that pertain to different stages in its life cycle, including an individual's age and size when it reproduces for the first time, offspring size and number, and many other traits (Stearns 1976). Much of life history theory centers around the idea of trade-offs, which refer to how individuals partition a finite amount of energy and resources between different functions. For example, an individual could produce and care for a small number of offspring or simply produce many offspring without offering parental care – but likely not both, as producing and caring for a large brood may be too costly (Zera & Harshman 2001). Life history traits typically vary according to the surrounding environment,

including the amount of resources available, predictability of the climate and predators, presence of toxins, and many other environmental features (Congdon et al. 2001).

Life history theory has given rise to the pace-of-life syndrome (POLS) hypothesis, which posits that other physiological and behavioral traits should covary in predictable ways with life history traits (Dammhahn et al. 2018). Under the pace-of-life hypothesis, individuals exist on a continuum of “slow” to “fast” life history strategies, where “fast” individuals will have higher metabolisms, exhibit riskier behaviors, and will mature earlier than “slow” individuals (Polverino et al. 2018). Ultimately, the POLS hypothesis could help explain how variation is maintained in populations; for example, density-dependent selection, in which a phenotype’s relative frequency determines its fitness, could mediate the persistence of fast and slow life histories within a population (Wright et al. 2019).

Maintenance of Melanism in Wild Populations

Typical male mosquitofish are silver-colored, but a minority of males – approximately one percent overall, but approximately 20% in some populations – exhibit a melanistic phenotype with black-and-white mottled scales (Horth 2006). This coloration is correlated with increased aggression toward other males and toward females (Horth 2003). Additionally, melanic males are larger than silver males and possess larger genitalia relative to body size (Horth et al. 2010). In some populations, male mosquitofish with the melanism allele will only express it (in both color and behavior) if they develop at lower temperatures; in others, expression of melanism is consistent across temperatures (Horth 2003, 2006). The gene for melanism appears to be Y-linked, even though melanic females occur rarely; based on crosses between melanic females and males, the melanic females are likely results of atypical sex determination, and still possess a Y-chromosome with the melanic trait (Angus 1989).

The mechanisms behind the persistence of the melanic phenotype in nature are varied. Melanic males possess a survival advantage over silver males in areas with predators; largemouth bass, dragonflies, and crawfish are all more likely to attack silver males than melanics (Horth 2004). Sexual selection may play a role in persistence of the trait, but whether the role is positive or negative can vary, and the degree of its effects against males’ forced copulation attempts are not clear. Females from populations without melanics prefer silver males, while females from populations with melanics prefer melanic males; even when reared

amongst melanics only, female offspring of the former population still preferred silvers (Bisazza & Pilastro 2000). However, females also tend to avoid melanics more often, although this may be in response to melanics' higher copulation attempts and the potential resulting injuries from their hooked genitalia (Horth 2003).

Melanic mosquitofish can and do out-compete silver males, but their aggression may limit their frequency in the population as well. Melanics display aggression toward silver males, often winning due to their larger size, and they may kill silver competitors for females (Horth et al. 2010). However, populations with high numbers percentages of melanic males also experience higher death rates of both melanics and females, potentially limiting the benefits of their mating aggression (Horth & Travis 2002). One study also found that melanics generally interact with more social partners, and more female social partners in particular, than silver males (Kraft et al. 2016). Beyond these factors, other varying traits may affect the persistence of melanism in populations.

This study aimed to determine how the pace-of-life syndrome hypothesis might apply to the persistence of variation within wild *G. holbrooki* populations. Given that melanic males exhibit both larger body size and higher aggression, both traits associated with faster paces of life, the two phenotypes could possess other life history differences. We specifically examined metabolic rate and cognitive flexibility to determine if variation in either trait could be an additional factor to explain how the rare melanistic phenotype is maintained.

Collection & Husbandry

Adult *G. holbrooki* were collected from Newport Spring, FL and the Wacissa River, FL in May 2019. Collected individuals were transported back to the Evolutionary Ecology Lab at UAH, where they were housed in 10-gallon tanks with filters and plastic rope plants for environmental enrichment. Each tank contained two silver males, two melanic males, and four females, all from the same original population. Tanks were fed twice *ad libitum* with flake food or frozen blood worms on weekdays or once on weekends. The tanks were maintained on a 12-hour photoperiod and around 75°F throughout the testing period from October 2020 to March 2021. Husbandry and experimental methods for this study were approved in IACUC protocols 2018.R006 & 2021.R01.Culumber.LivebearingFishes.

Metabolic Rate

Significance

Metabolic rates are often considered the key physiological feature underpinning life history and pace of life (POL); variation in metabolic rate can be intimately tied with POL variation (Auer et al. 2018), and metabolism is often considered a main feature determining other life history characteristics like immunity (Jung et al. 2019) and maturation rate (Gangloff et al. 2020). Here, we measured standard metabolic rates (SMR) to determine if variation in these rates between silver and melanic *G. holbrooki* males exists. If the two phenotypes differ in their metabolic rates, then this variation could indicate the presence of POL differences that may aid in the persistence of the rare melanistic phenotype in wild populations.

Methods

Experimental methods were adapted from Culumber 2016. A total of 22 males from the Newport Spring, FL population, including 11 of each color phenotype, were tested for their standard metabolic rates by measuring their oxygen consumption in a closed container. Individuals were fasted for 24 to 36 hours prior to testing to ensure each one was in a post-absorptive state (i.e., not actively digesting food, when metabolic rates are elevated) during measurement. For acclimation on the evening before testing, each male was placed in an opaque 500 mL plastic Nalgene bottle containing approximately 350 mL of conditioned water and a lightly-bubbling air stone to maintain consistent dissolved oxygen levels. Five bottles were tested per round of trials, with one bottle containing no fish to measure background respiration rate by any microbes present in the water. The bottles were placed into a 10-gallon water bath filled to a 10 cm depth with conditioned water, an Eheim-Jäger 150W aquarium heater set to 77°F, and a vigorously-bubbling air stone to ensure even temperature throughout the bath. For their acclimation period, bottled individuals were left in this water bath overnight for at least 16 hours.

Following acclimation, the air stones in each bottle were removed. Each bottle was flushed and filled completely with conditioned water. Bottles were capped tightly, and a calibrated YSI ProSolo optical dissolved oxygen probe was inserted through the lid of each bottle and sealed in with plumber's putty to ensure no gas exchange with the surrounding air

during the trial. The probes were allowed to record oxygen levels inside the bottles for at least four hours.

After recording was completed, each individual's blotted dry mass was measured before being returned to their tank. Bottles and lids were sterilized with a 10% bleach solution to ensure no microbes accumulated inside the bottles between trials and subsequently rinsed prior to the next trial.

Statistical Analyses

I used an analysis of covariance (ANCOVA) to evaluate variation in SMR between melanic and silver males. To estimate metabolic rate, the first 90 minutes of dissolved oxygen (DO) readings were discarded and a linear regression was conducted between the remaining DO values and time. The regression slopes were used as estimates of SMR for each individual. The SMR data were used as the dependent variable in the ANCOVA along with fixed effects of male color (melanic or silver), body size, and temperature. We also included a random effect of tank to account for potential differences in the social environment.

Results

The melanic males averaged a mass of 0.349 ± 0.017 g (\pm SE), while the silver males averaged 0.297 ± 0.030 g. Since oxygen depletion rates in the empty bottles were relatively consistent across trials, the metabolic rate measurements were not adjusted for background respiration. Regression slopes indicated that melanic males consumed dissolved oxygen at a slightly greater rate (-0.000141 ± 0.000015) than silver males (-0.000130 ± 0.000013). However, these color phenotypes did not vary significantly in their standard metabolic rates ($F=2.199$, $p=0.162$; fig. 2).

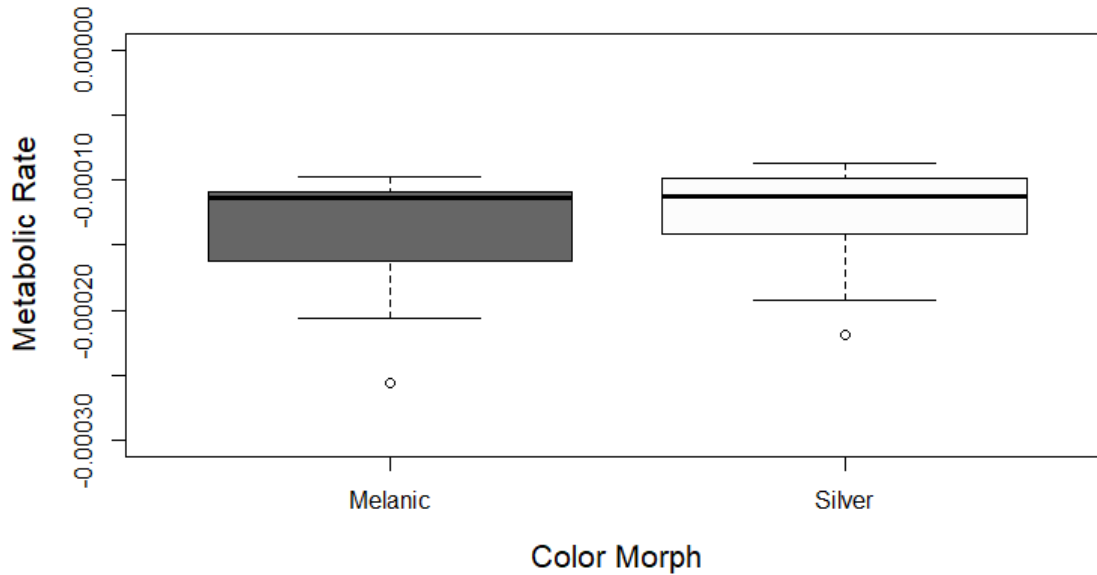


Figure 2 – Standard metabolic rate (unitless, calculated by regression) for melanic and silver color phenotypes of male *Gambusia holbrooki*.

The home tank and body mass of each individual did not have any significant effect on metabolic rate ($F=2.458$, $p=0.089$; $F=1.335$, $p=0.269$, respectively). Temperature, however, did significantly affect measured metabolic rates ($F=5.136$, $p<0.05$).

Discussion

Our results indicate that melanic and silver male *G. holbrooki* do not differ in their standard metabolic rates. While the two phenotypes tend to vary in some pace-of-life traits, including body size (Horth et al. 2010) and aggression (Horth 2003), research has not yet characterized broad physiological differences, such as variation in immunity or hormone levels, that should be expected if life history traits vary between the morphs. Broader experiments that measure more life history traits may be necessary to uncover significant trends, especially given the variation in how well different species abide by the predictions of the POLS hypothesis (Royauté et al. 2018).

Additionally, only standard metabolic rate near the populations' typical home temperature was measured in this experiment. A previous study with bluegill sunfish (*Lepomis macrochirus*) found a relationship of life history with metabolic scope measurements rather than with standard metabolic rate alone (Binder et al. 2016). Measurements of metabolic scope for

both color phenotypes, where maximum metabolic rate is measured after exercise and/or metabolic rates are measured at different temperatures, could uncover important differences between these morphs with potential roles in the persistence of the rare melanistic phenotype.

The insignificance of each individual's tank reflects a general homogeneity of social environments and ambient conditions across all tanks, so the SMR measurements were not strongly affected by differences in social partners or the environment of their home tank. However, the lack of effects from body mass are more surprising; generally, metabolic rates will increase with body mass (Clarke & Johnston 1999). Here, the comparatively small sample size with a comparatively low range of body sizes may obscure any predictive effects of mass on metabolic rates in *G. holbrooki*.

Finally, metabolic rate tends to increase with temperature in both fishes (Clarke & Johnston 1999) and broadly across taxa, including both endothermic and ectothermic species (Gillooly et al. 2001). Likewise, the significant effect of temperature on these measured metabolic rates in *G. holbrooki* fits this trend.

Cognitive Flexibility

Significance

Cognitive flexibility refers to an individual's ability to navigate new and/or complex environments or tasks. Flexibility can affect how individuals learn from others, such as learning foraging behavior from tutors (Fuss et al. 2021), and it can impact how well individuals can navigate their environment (Croston et al. 2017). Cognitive abilities have been linked with life history traits; in black-capped chickadees (*Poecile atricapillus*), fast explorers in new environments exhibited worse learning than slower explorers (Guillette et al. 2011). Here, we measured cognitive flexibilities to determine if variation in cognitive ability between silver and melanistic *G. holbrooki* males exists. If the two phenotypes differ in their cognition, then this variation could indicate the presence of POL differences that may aid in the persistence of the rare melanistic phenotype in wild populations.

Methods

A total of 47 individuals from the Newport Spring and Wacissa River, FL populations, including 24 melanistic and 23 silver males, were tested for their cognitive flexibilities with a detour task. Experimental methods were adapted from Wallace et al. 2020. One focal male was placed into a tank, filled to a 10 cm depth with conditioned water, in which he would need to navigate out of a corridor and around a transparent barrier to reach a reward female (fig. 3).

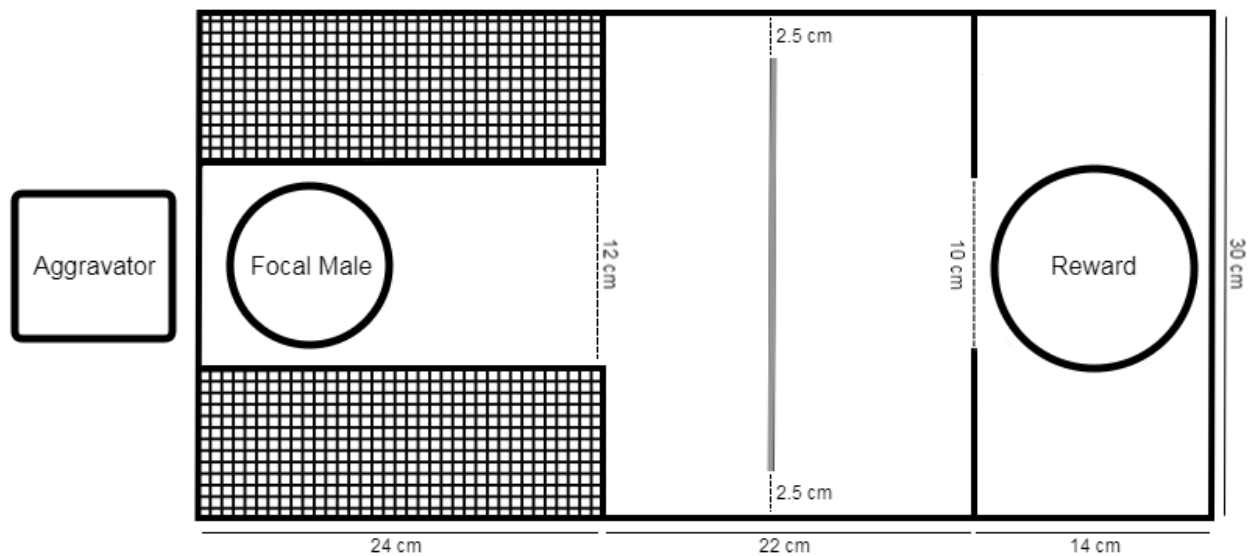


Figure 3 – Diagram of the detour task tank. The grey vertical line in the center marks the location of the transparent barrier that each male needed to navigate around to reach a reward female, who was visible on the opposite end of the tank.

The focal male was placed in an opaque tube at the end of a large tank, where he was allowed to acclimate for five minutes to minimize any effects from stress after transport between tanks. A reward female was placed in a transparent cup at the opposite end, and an aggravator male was placed in a separate transparent container beside the focal male's starting point. Both the reward female and aggravator male were from drawn randomly from different tanks and populations than the focal male to ensure no previous social interactions between individuals would affect performance. Aggravators were exclusively silver males regardless of the focal male's phenotype. After five minutes, the acclimation tube was lifted, and the focal male was left to try and navigate out of his starting corridor and around the transparent barrier between himself and the reward female. The time taken to reach the clear barrier and, afterward, to enter the reward area were recorded for each male.

Regardless of whether the focal male successfully completed the detour task, he was removed after 10 minutes and photographed to measure body lengths before being returned to his home tank. 20% of the water in the detour task tank was replaced after every second trial to ensure no buildup of waterborne stress hormones or microbes across trials. The reward female and aggravator male were used for two trials in a row, one of each color phenotype, before being removed and photographed as well. The total time it took the focal individual to reach the reward

female was considered a measure of motivation. The time from reaching the barrier to completing the task was used as the measure of cognitive flexibility. Individuals that did not complete the task were assigned a maximum value of 600s.

Standard body lengths of focal males, reward females, and aggravator males were all measured from photographs by using ImageJ (Rasband 2021). Body length measurements did not include the caudal tail fin.

Statistical Analyses

Analyses generally followed those described for metabolic rate. ANCOVA was used with the score of cognitive flexibility as the dependent variable with fixed effects of male color and body size. A random effect of tank was also included. Lastly, I included covariates for the size of the aggravator male and the reward female as they could influence motivation or the time taken to complete the detour task.

Results

The melanic males took an average of 245 ± 42.1 s (\pm SE) to complete the detour task, while the silver males took 345 ± 52.6 s. These color phenotypes did not vary significantly in their cognitive flexibilities ($F=2.262$, $p=0.143$; fig. 4).

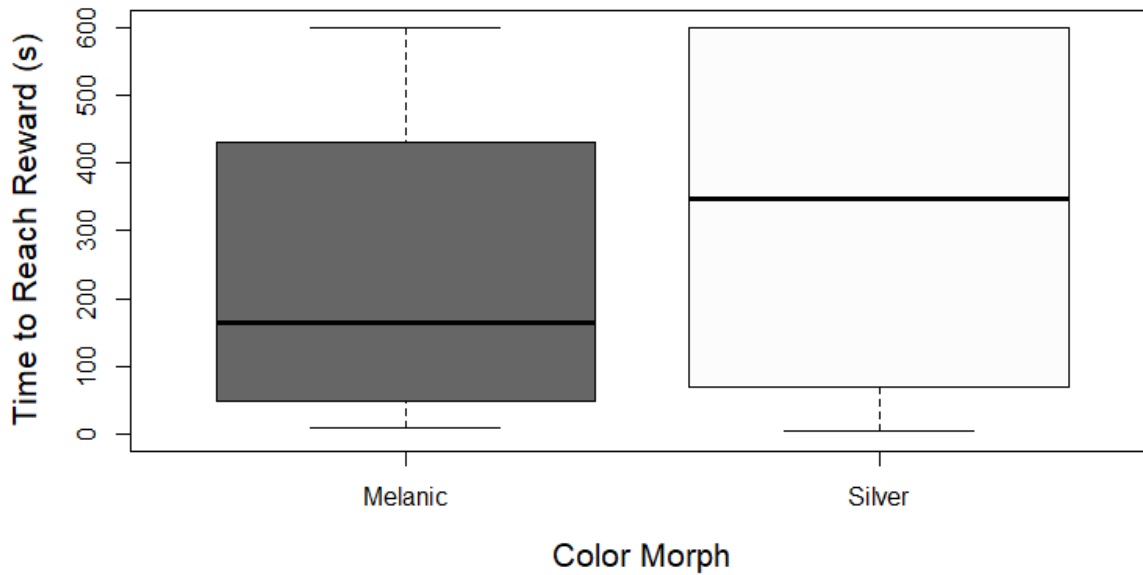


Figure 4 – Time elapsed (s) for two color phenotypes of male *Gambusia holbrooki* to complete a detour task. This score reflects cognitive flexibility - an individual's ability to change its behavior according to its current environment.

Home tank and body length of the focal male did not significantly affect his performance ($F=1.068$, $p=0.420$; $F=3.071$, $p=0.09$, respectively). The body length of the reward female was also not a significant predictor of performance in the detour task ($F=0.261$, $p=0.613$). Interestingly, however, the body length of the aggravator male was a significant predictor ($F=9.081$, $p<0.01$). Finally, the time taken to reach the transparent barrier also affected individuals' speed in completing the detour task ($F=8.195$, $p<0.01$).

Discussion

These two color phenotypes did not differ significantly in their cognitive flexibilities. Given the relatively low spatial complexity of typical *G. holbrooki* habitats, which generally include still or slow-flowing water (Froese & Pauly 2010), selection for cognitive flexibility may be low for most populations. As such, few differences between individuals would be expected.

The insignificance of home tank and body length are unsurprising. As was true in the SMR experiment, the social environment and ambient conditions in each tank did not affect scores. Likewise, the ratio of brain to body size in fishes can vary across size classes, and in cleaner fishes (*Labroides dimidiatus*), body size did not predict cognitive performance (Triki et

al. 2021). In *G. holbrooki* males, brain size may not impact cognition heavily, especially if flexibility is under weak selection within wild populations.

The lack of significance for reward female size was much more surprising. Male *G. holbrooki* have demonstrated preferences for larger female mates (Bisazza et al. 1989; Hoysak & Godin 2007). However, given the high significance of aggravator male length, the focal males may have been influenced more heavily by competition than by their typical mate choice. In some other poeciliid fishes, larger males tend to outcompete small males (Bisazza 1993), so aggravator size may have had a stronger effect in driving focal males across the tank than initially expected. Further research on the effects of male-male competition in *G. holbrooki* could illuminate the degree to which size influences male behavior.

Finally, the significant relationship between focal males' time to barrier and time to completion is unsurprising. Reaching the barrier quickly meant that the focal male could start navigating around it sooner and would thus be more likely to complete the detour task quickly.

Conclusion

The results of these two experiments do not support the pace-of-life syndrome hypothesis among male *Gambusia holbrooki*. Males with the rare melanistic phenotype did not differ from their common silver counterparts in standard metabolic rate or cognitive flexibility. These two traits are unlikely to be major contributors to the maintenance of the melanistic phenotype within wild populations.

Further research using different measures of metabolism could better characterize any possible differences between the color phenotypes. In bluegill sunfish, only metabolic scope covaried with boldness (Binder et al. 2016), and in killifishes, measurements of standard MR, maximum MR, and metabolic scope differed in their capacity to explain POL variation across species (Methling et al. 2020). Measurements of maximum metabolic rate and metabolic scope may indicate clearer links between metabolism and other life history traits in *G. holbrooki*, if present.

The high significance of aggressor male size during the detour task also presents an interesting avenue for future research. While the preference for larger mates in male *G. holbrooki* has been characterized (Bisazza et al. 1989; Hoysak & Godin 2007), the interactions between males' size and behavior have not been studied yet. In groups of *G. holbrooki*, the largest male tends to monopolize access to females, and these larger males behave aggressively to prevent smaller males from mating with females (Bisazza & Marin 1995). Research examining the smaller males in these social groups, particularly their dispersal and reproductive behaviors, could illustrate how body size influences males' social interactions and behaviors. Since silver males tend to be considerably smaller than melanistic males, these behaviors could also indicate how differences in behavior and mating success affect the persistence of the melanistic phenotype.

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