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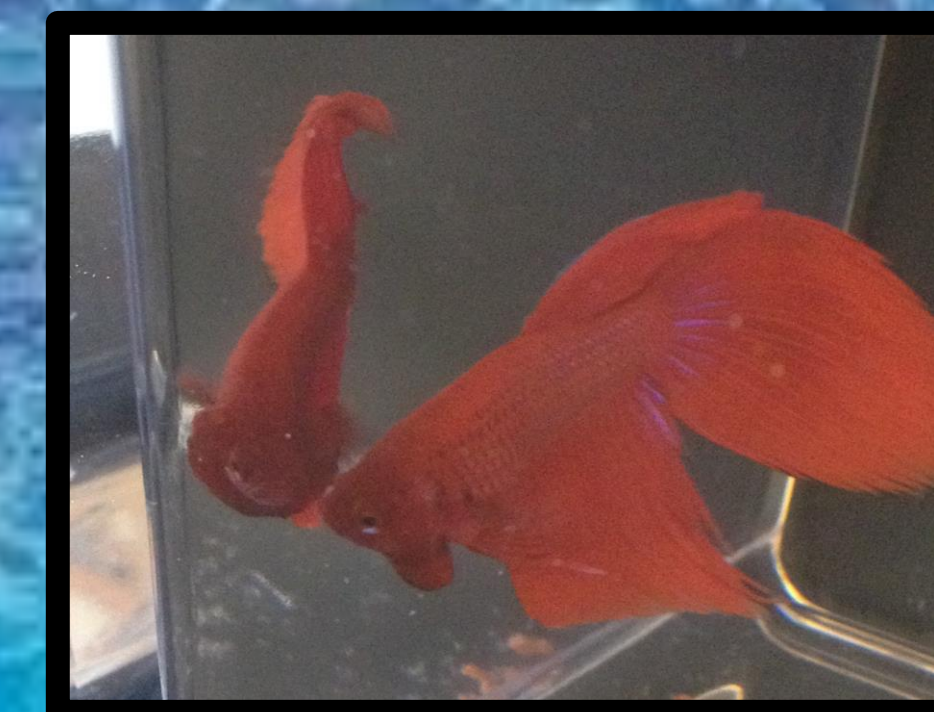
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Aggressive Displays in Male *Betta splendens* Based on Color Patterns

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A male *B. splendens* flaring its gills and fins at a reflection.

Abstract

The traits of an opponent can influence how an individual uses aggressive displays to dissuade the opponent from physical conflict. Whereas one previous study found that conspecific aggression was less intense between differently-colored Siamese fighting fish (*Betta splendens*), we felt that more evidence was needed to corroborate or dispute their findings. We hypothesized that *B. splendens* act less aggressively towards similarly-colored fish than to differently-colored fish, based on the notion that males would avoid conflict with a phenotypically similar opponent due to the costs of displaying. To test for differences in aggression due to coloration, we paired 10 fish in view of one another, as well as in view of a mirror reflection, and measured duration of gill flaring and the number of tail beats. We found that the duration of gill flaring and the number of tail beats were significantly lower between differently-colored pairs of fish compared to the other groups. While contrary to our predictions, our data corroborate previous studies on color bias in aggressive displays. Our findings indicate that, as with other species, coloration in *B. splendens* may influence interactions due to underlying phenotypic influences.

Introduction

Aggressive displays play a significant role in both intra- and interspecific interactions, such as territory defense, by conveying the displaying individual's fighting ability, which can dissuade an opponent from combat (Hess et al. 2016). These displays can also be metabolically costly (Arnott, 2016; Castro, et al., 2006), so the displayer must appraise the opponent to avoid exhaustion, physical harm, or death.

Siamese fighting fish (*Betta splendens*) use conspicuous aggressive displays (i.e., tail beating and flared gill covers), which makes them an ideal species for studying aggression (Abante, 2005). One study analyzed color bias in aggression between male *B. splendens* and found that males used more aggressive displays towards similarly-colored individuals than towards differently-colored males (Thompson & Sturm, 1965). Another study found that aggressive displays in *B. splendens* are not significantly different when directed at a reflection or at an opponent (Arnott et al., 2016). We wanted to conduct an experiment to corroborate or refute color bias in *B. splendens* aggression, so we tested whether coloration influences aggressive displays between male *B. splendens*.

Objective: To see if a difference in aggressive displays occurs between similarly-colored fish, differently-colored fish, and fish towards their reflection. A significant difference in aggressive displays between treatments may indicate a color bias in aggression.

Predictions: More tail beats and longer gill flaring will occur between differently-colored fish than between similarly-colored fish. Aggressive displays directed at a reflection will be at a similar rate as displays between similarly-colored fish. Our predictions are based on previous research which found no significant difference in displays directed at a reflection compared to those directed at an opponent (Arnott, et al., 2016)

Methods and Materials

Conditions - We kept 10 male veiltail *B. splendens* (5 mostly blue and 5 mostly red) in 160 Science at the University of Southern Maine. We placed the fish in 5 1.90L tanks, each with a divider installed in the middle of the tank. We also placed cardboard dividers around the tanks so fish could not see each other. The fish were fed once daily, and we conducted our experiment twice weekly, with at least 3 days between trials to avoid fatiguing the fish.

Procedure - During each session, we randomly selected and paired 8 fish. Each pair of fish had their tanks pushed together, with dividers placed between the test fish (Fig. 1). We then removed the divider between test fish and began a 5-min. observation period. We observed both test fish, counting tail beats over 30-s intervals and, using stopwatches, recording the duration of gill flaring throughout the trial. One focal fish was randomly selected per trial for data analysis. We positioned the two remaining fish in front of a mirror with a divider in front of it, and recorded the same data.

Analysis - We used ANOVA & post hoc t-tests to compare the number of tail beats and duration of gill flaring between differently-colored pairs of fish, similarly-colored pairs, and a fish towards its reflection. The coloration of opponents served as our independent variable, while the number of tail beats and the duration of gill flaring seen from the fish served as our dependent variables. We used JMP 12.2 (SAS Institute, Inc., 2015) for analysis, with a significance level of 95% ($p \leq 0.05$).

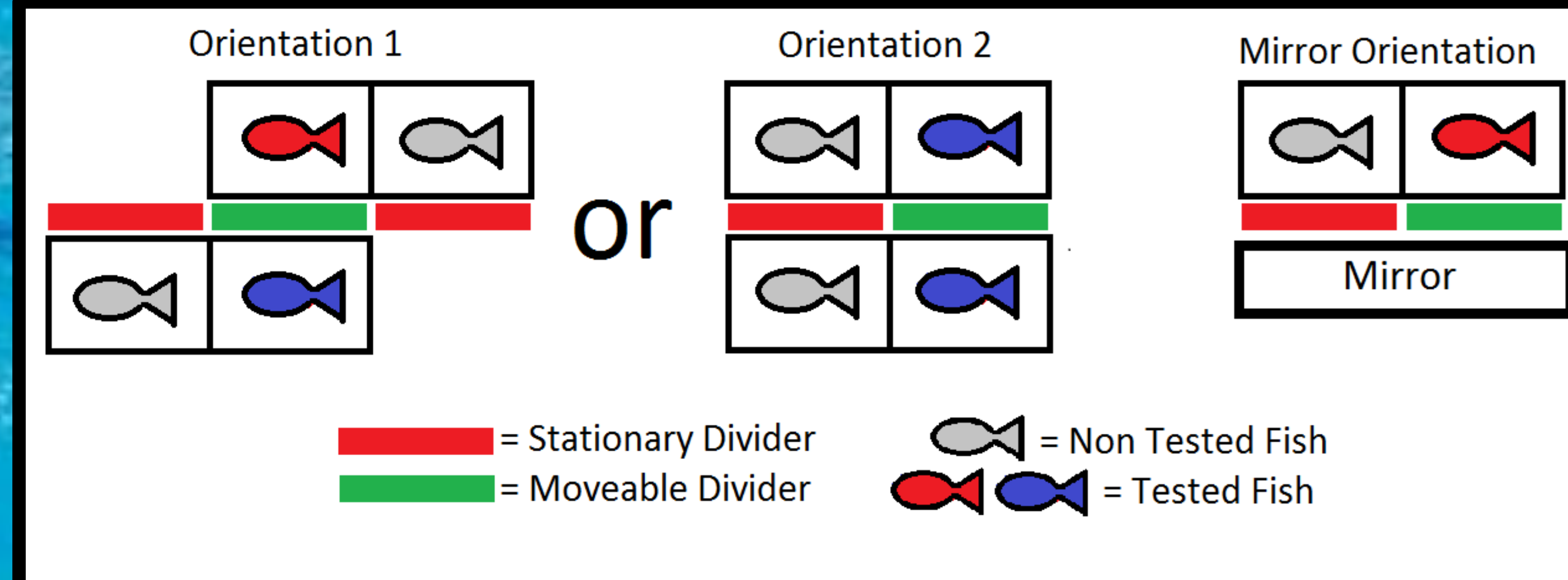


Figure 1. Tank orientations and divider placement for trials between 2 male *B. splendens*, as well as mirror trials.

Results

- There was a significant difference between treatments for both the duration of gill flaring ($F = 6.36$, $df = 2$, $p = 0.008$; Fig. 2) and the number of tail beats ($F = 4.76$, $df = 2$, $p = 0.022$; Fig. 3).
- The duration of gill flaring in the different color group was significantly lower than the durations in both the same color and the reflection groups ($p < 0.05$). The difference between the same color and mirror groups was not significant ($p > 0.05$).
- The number of tail beats in the different color group was significant lower than the number in the mirror group ($p < 0.05$). The differences between the same color group and both the different color and the reflection groups were not significant ($p > 0.05$).

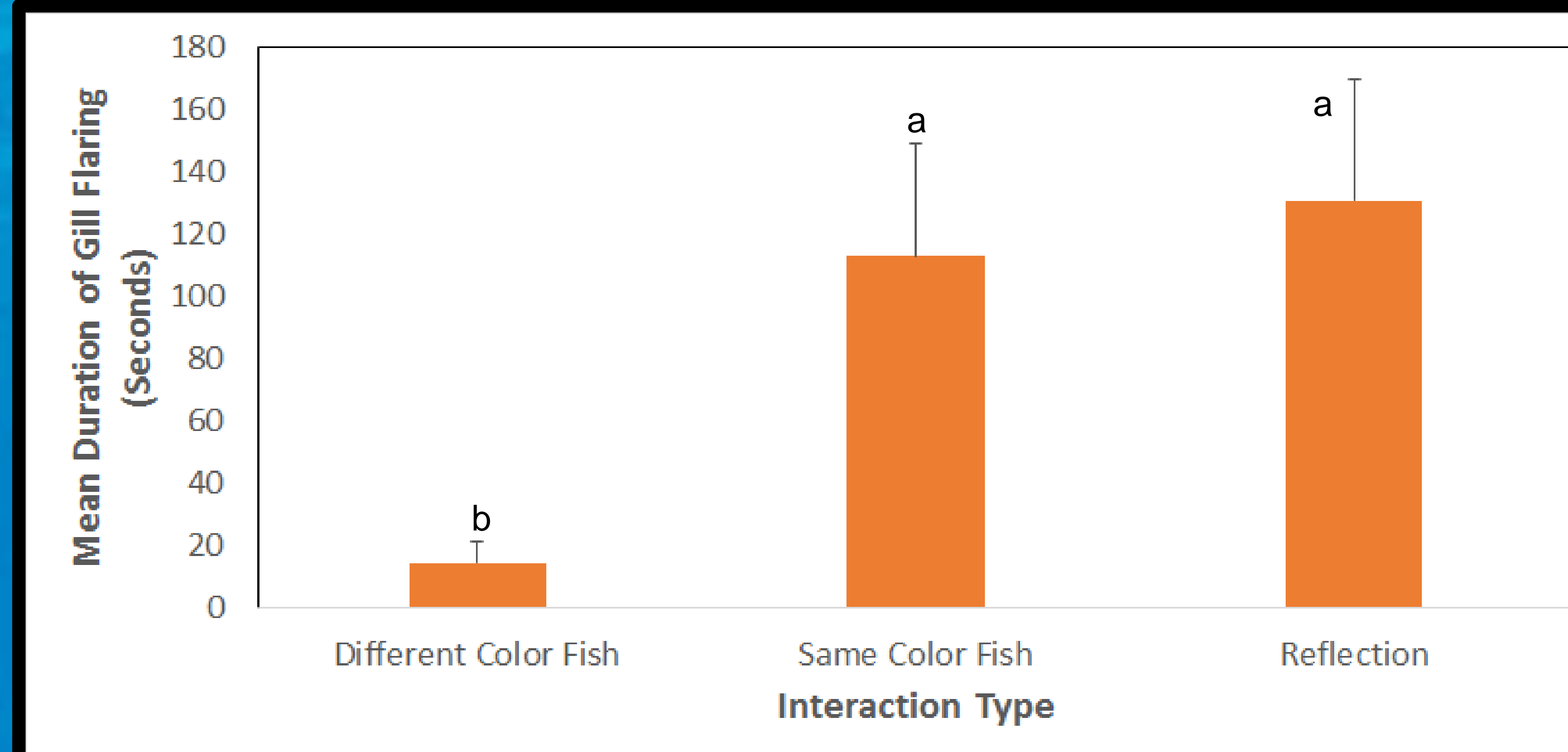


Figure 2. Mean (+ SD) of the duration of gill flaring between the *Betta splendens* display groups. Different letters by bars indicate statistically significant differences between groups.

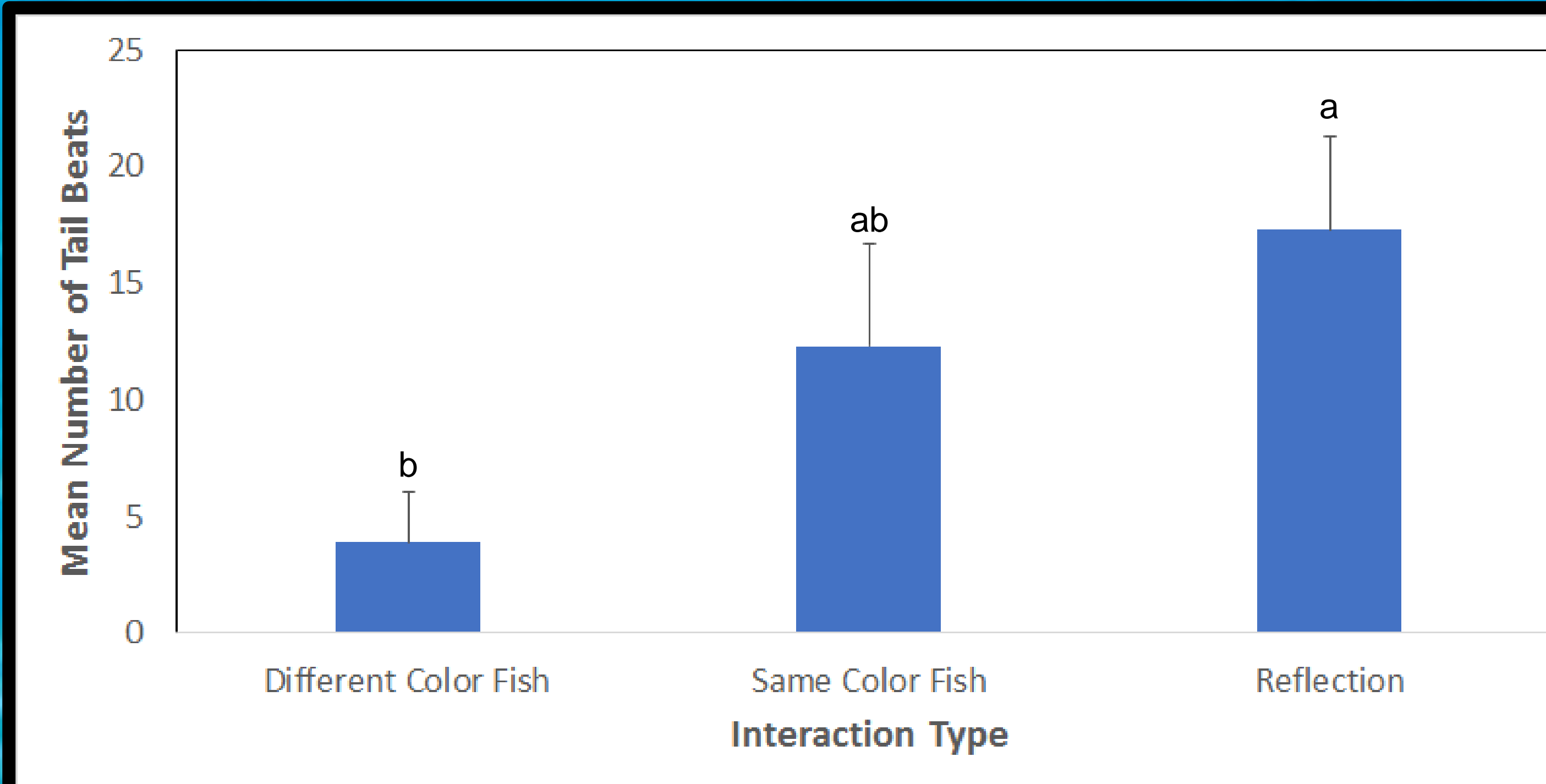


Figure 3. Mean (+ SD) of the number of tail beats between the *Betta splendens* display groups. Different letters by bars indicate statistically significant differences between groups.

Discussion

Gill flaring in the differently-colored male *B. splendens* was significantly shorter in duration than gill flaring in both the same color and mirror pairings, which refutes our initial predictions that gill flaring duration would be shorter in the same color pairs than in the different color pairs. While the number of tail beats was less in the different color pairs than the same color pairs, the difference was not statistically significant. The number of tail beats in the same color pairing was also non-significantly lower than displays between a fish and their reflection. The unusually low rate in the same color group may be due to early submittance in some of the same color pairings, so additional trials with a larger sample size could improve the results of tail beat number between groups.

B. splendens don't recognize their reflection, at least in terms of displaying, as indicated by the non-significant difference between the mirror and same color pairs for both tail beats and gill flaring. The measured difference in both display types between the same color pairs and the mirror group could be due to heightened aggression from the mirror group towards a more aggressive pseudo-opponent. Since the perceived opponent does not stop displaying until the displaying fish surrenders, the aggression between the displayer and its reflection could last longer than aggression between two fish when either opponent can surrender before the other.

Our experiment indicates that male *B. splendens* use less aggressive displays towards differently-colored males than similarly-colored males, which supports previous research on aggression between different color types (Lehtonen, 2014; Pryke & Griffith, 2006; Thompson & Sturm, 1965). Researchers attributed these differences in aggression to underlying influences of phenotypic diversity of coloration in a population (Lehtonen, 2014). In some species, certain color types can assert dominance over some color types more quickly than other color types by initiating aggression sooner or more often (Pryke & Griffith, 2006). This assertion of dominance may also apply to male *B. splendens*: one color type may be asserting dominance over the other color type more quickly, ending aggressive interaction sooner in the different color pairing than in the same color pairing.

Due to the lower levels of aggression witnessed between *B. splendens* of different colors, the possibility that male *B. splendens* can share a tank with other males so long as they are of different colors may be present, though further research is needed to support or reject this notion.

Coloration in male *B. splendens* does appear to impact conspecific aggression, though the dynamics of color type influence in *B. splendens* is currently unclear. Our findings could open the door for further investigation into the significance of color type in *B. splendens* interactions and in the interactions of other species.

Acknowledgements

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