A Study of Female Courtship Behavior and Mating Preferences in *Betta splendens*

Abby Brownell

*Lake Forest College, brownap@lakeforest.edu*

Follow this and additional works at: [http://publications.lakeforest.edu/seniortheses](http://publications.lakeforest.edu/seniortheses)

Part of the [Biology Commons](http://publications.lakeforest.edu/seniortheses)

Recommended Citation

A Study of Female Courtship Behavior and Mating Preferences in *Betta splendens*

Abstract

Siamese fighting fish, or *Betta splendens*, are known for their flashy colors and extremely aggressive behavior. They are a perfect model species for studying sexual behavior. First, I reviewed betta mate choice and the courtship ritual. Female betta are known to select a male based on a number of visual cues such as male size, vigor, fin length, and aggression in addition to male territory and bubble nest quality. Then I examined what mattered most to the female: movement or coloration. I studied this phenomenon in captive bred betta fish and found that females prefer red colored males over blue coloration in males. However, when red males were anesthetized, female betta preferred blue colored males over red colored males. This preference for red coloration may reflect the role of carotenoid dependent signaling.

Document Type

Thesis

Distinguished Thesis

Yes

Degree Name

Bachelor of Arts (BA)

Department or Program

Biology

First Advisor

Anne Houde

Second Advisor

Douglas Light

Third Advisor

Elizabeth Fischer

Keywords

*Betta splendens*, Siamese fighting fish, sexual behavior, coloration

Subject Categories

Biology

This thesis is available at Lake Forest College Publications: http://publications.lakeforest.edu/seniortheses/42
Lake Forest College Archives

Your thesis will be deposited in the Lake Forest College Archives and the College’s online digital repository, Lake Forest College Publications. This agreement grants Lake Forest College the non-exclusive right to distribute your thesis to researchers and over the Internet and make it part of the Lake Forest College Publications site. You warrant:

- that you have the full power and authority to make this agreement;
- that you retain literary property rights (the copyright) to your work. Current U.S. law stipulates that you will retain these rights for your lifetime plus 70 years, at which point your thesis will enter common domain;
- that for as long you as you retain literary property rights, no one may sell your thesis without your permission;
- that the College will catalog, preserve, and provide access to your thesis;
- that the thesis does not infringe any copyright, nor violate any proprietary rights, nor contain any libelous matter, nor invade the privacy of any person or third party;
- If you request that your thesis be placed under embargo, approval from your thesis chairperson is required.

By signing below, you indicate that you have read, understand, and agree to the statements above.

**Printed Name:** Abby Brownell

**Thesis Title:** A Study of Female Courtship Behavior and Mating Preferences in *Betta splendens*

This thesis is available at Lake Forest College Publications: [http://publications.lakeforest.edu/seniortheses/42](http://publications.lakeforest.edu/seniortheses/42)
LAKE FOREST COLLEGE

Senior Thesis

A Study of Female Courtship Behavior and Mating Preferences in Betta splendens

by

Abby Brownell

December 5, 2014

The report of the investigation undertaken as a Senior Thesis, to carry two courses of credit in the Biology Department

Michael T. Orr
Krebs Provost and Dean of the Faculty

Anne Houde, Chairperson

Douglas Light

Elizabeth Fischer
Abstract

Siamese fighting fish, or *Betta splendens*, are known for their flashy colors and extremely aggressive behavior. They are a perfect model species for studying sexual behavior. First, I reviewed betta mate choice and the courtship ritual. Female betta are known to select a male based on a number of visual cues such as male size, vigor, fin length, and aggression in addition to male territory and bubble nest quality. Then I examined what mattered most to the female: movement or coloration. I studied this phenomenon in captive bred betta fish and found that females prefer red colored males over blue coloration in males. However, when red males were anesthetized, female betta preferred blue colored males over red colored males. This preference for red coloration may reflect the role of carotenoid dependent signaling.
For my family, both human and four legged
Acknowledgments

First, I would like to thank Anne Houde for giving me the opportunity to work in her lab. Her constant guidance, support, and encouragement over the last year has made this thesis possible. She provided the perfect amount of independence and support, which has allowed me to grow as a student and a scientist. I would also like to thank Margaret Frank for her assistance with my statistics. I would not have been able to complete this without both of you!

Secondly, I would like to thank my family and friends for their unwavering support. Without them, I would have never made it this far. I would especially like to thank my parents for all of their help over the last four years. I would never have been this successful if it had not been for their unwavering love and tolerance for late night phone calls.

Finally, I would like to thank Lynn Westley for being the most influential and supportive person I have met at Lake Forest College. Without her I would not have had the courage to follow my dreams or the opportunity to further my education after I graduate.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures</td>
<td>i</td>
</tr>
<tr>
<td>List of Tables</td>
<td>ii</td>
</tr>
<tr>
<td>Introduction to the Betta</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 1. A Review of Betta Mate Selection and Courtship Behavior</td>
<td>2</td>
</tr>
<tr>
<td>Mate Selection</td>
<td>4</td>
</tr>
<tr>
<td>Courtship Behavior</td>
<td>8</td>
</tr>
<tr>
<td>Chapter 2. Female mating preferences as a result of coloration and movement in <em>B. splendens</em></td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>14</td>
</tr>
<tr>
<td>Methods I</td>
<td>18</td>
</tr>
<tr>
<td>Results I</td>
<td>22</td>
</tr>
<tr>
<td>Methods II</td>
<td>23</td>
</tr>
<tr>
<td>Results II</td>
<td>25</td>
</tr>
<tr>
<td>Methods III</td>
<td>28</td>
</tr>
<tr>
<td>Results III</td>
<td>30</td>
</tr>
<tr>
<td>Methods IV</td>
<td>32</td>
</tr>
<tr>
<td>Results IV</td>
<td>33</td>
</tr>
<tr>
<td>Discussion</td>
<td>35</td>
</tr>
<tr>
<td>References</td>
<td>40</td>
</tr>
<tr>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Figure 1. Male and female images</td>
<td>3</td>
</tr>
<tr>
<td>Figure 2. Experiment I apparatus</td>
<td>19</td>
</tr>
<tr>
<td>Figure 3. Female color preference by trial count</td>
<td>22</td>
</tr>
<tr>
<td>Figure 4. Female color preference by mean time</td>
<td>23</td>
</tr>
<tr>
<td>Figure 5. Experiment II apparatus</td>
<td>25</td>
</tr>
<tr>
<td>Figure 6. Male response to anesthetization using MS-222</td>
<td>26</td>
</tr>
<tr>
<td>Figure 7. Male daily time spent engaged in behavior pre-anesthetization</td>
<td>27</td>
</tr>
<tr>
<td>Figure 8. Experiment III apparatus</td>
<td>29</td>
</tr>
<tr>
<td>Figure 9. Time ratio for each of the four trial conditions</td>
<td>31</td>
</tr>
<tr>
<td>Figure 10. Total mean time for each of the four trial conditions</td>
<td>32</td>
</tr>
<tr>
<td>Figure 11. Experiment IV apparatus</td>
<td>33</td>
</tr>
<tr>
<td>Figure 12. Time ratio for cutout models in each of the four trial conditions</td>
<td>34</td>
</tr>
<tr>
<td>Figure 13. Total mean time for cutout models in each of the four trial conditions</td>
<td>35</td>
</tr>
<tr>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Table 1. The categorized behavior witnessed during trials by male and female betta</td>
<td>21</td>
</tr>
<tr>
<td>Table 2. Observed behavior for each level of vigor</td>
<td>21</td>
</tr>
<tr>
<td>Table 3. R² and slope values for male betta pre-anesthetization</td>
<td>28</td>
</tr>
</tbody>
</table>
Introduction

Behavior is constantly being modified not only during an individual's life, but throughout a countless number of generations. During this time, species adapt behaviorally and genetically to their changing environment. The fitter individuals survive and pass along their genes to subsequent generations, and in time the frequency of alleles in the population can change. This is the basis for the evolution of present day traits in a population. Ethology is the study of an organism’s actions and seeks to explain them with respect to behavior and not as an abstract idea of the organism’s thought processes (Eibl-Eibesfeldt 1970). The field seeks to understand why behaviors occur, including learned versus innate mechanisms, and how environmental pressures lead to evolution of behaviors. Mating behavior in particular has been a topic of interest across a wide variety of species. Fish are often studied due to their vastly different and specific mating rituals (Hoar 1962). In addition, it is relatively easy to set up artificial environments in the laboratory and maintain large breeding populations for many species. One such species that is often studied is the domesticated Betta splendens.

In this thesis, I will first examine the courtship process of B. splendens and how mates are selected. This is important to understand because betta have a complex courtship ritual and mates are selected based on a number of visual and behavioral cues. From there, I will investigate the relationship between coloration and movement on mate selection when direct physical contact is denied to a domestic stock of betta.
Chapter One

A Review of Betta Mate Selection and Courtship Behavior

*B. splendens*, more commonly known as betta, are well known for their flashy colors (Figure 1) and extremely aggressive behavior (Doutrelant & McGregor 2000). Generally found in standing water in Thailand, Cambodia, and Vietnam, these fish have been commercially bred and sold for over a century. The fish bought in pet stores bear little physical resemblance to their wild ancestors, yet their mating behavior has remained relatively unchanged. As a result, they are an excellent model species for both intrasexual and intersexual selection. If placed in the same enclosure, male bettas will fight, especially if they are protecting a territory containing a bubble nest meant for a receptive female (Noble 1938). This competition between conspecific males for territory, females, and/or defense, is an example of intrasexual selection. Female betta also “eavesdrop” on fighting males to select the winner to mate with, which is an example of intersexual selection (Doutrelant & McGregor 2000). Another example of intersexual selection occurs when either sex selects a mate. A male will attack a female to determine her readiness to breed, thus determining if she is receptive, fit, and worth the energy it takes to produce sperm and copulate (Noble 1938). The female must also ensure the same of the male, in addition to ensuring his territory and bubble nest are suitable for the offspring.

While the courtship behavior of *Betta splendens* is relatively well understood, there is still much to learn about mate selection. Various studies have examined the phenomenon known as the audience effect where an eavesdropping individual can obtain information that affects mate choice. If provided the opportunity, females will watch two
males fight over territory and select the winner with which to mate. In addition, male betta have even been observed watching two other males fight and then challenging the loser (Doutrelant et al. 2001). However, little is known about what visual cues assist a female in selecting a mate. In this literature review I will examine the various visual and behavioral cues that assist male and female betta in selecting a mate. Later, I will review the courtship ritual and its importance in the mating process.

Figure 1. Images of a red colored male, a blue colored male, and a female.
Mate Selection

Mate selection, or intersexual selection, has been a subject of interest since Charles Darwin first introduced his ideas on natural selection in 1859 and elaborated further on sexual selection in his 1871 book (Darwin 1859; Darwin 1871). Sexual selection is seen as being an important evolutionary agent because it affects morphology and behavior, in addition to being responsible for the current physiological traits of the species (Andersson, 1994). Darwin (1859) says that the theory of sexual selection seeks to explain the existence of traits, how these traits vary in the species, and how some traits are selected for or favored. When faced with the variability of traits among organisms, biologists turn to the theory of adaptation by natural selection, where the survival and wellbeing of a population or species is a primary focus. This theory has difficulty explaining the evolution of secondary sexual traits because individuals do not select mates for the survival advantage of the individual, but rather base their selection on individual consequences for reproductive success (Emlen & Oring 1977). While there may be innate behaviors that assist the individual in making a selection that improves its evolutionary fitness, this is a subconscious process. A well-known example is the role of odor in human mate selection. Numerous studies have looked at this phenomenon and discovered that genetically compatible pairs report that they enjoy the scent, or pheromones, of their partner (Grammer et al. 2005; Wedekind 2000). While this selection method is possible for some species, others must resort to other cues. However, the overarching theme among species is that one sex is competitive (usually males) with members of the same sex while the other sex is selective (usually females) in picking a mate.
In *Betta splendens*, the females are the choosy sex while the males compete with other males for territory and mates. The audience effect is a well-documented phenomenon where a third-party individual observes the behavior of two interacting individuals. This is a low-risk way of obtaining information about the mating ability of the opposite sex or the fighting ability of the same sex (McGregor et al. 1993). The eavesdropping individual does not have to engage in a costly fight or an energetic mating ritual with an unsuitable mate and reduces the risk of escalated conflicts (Oliveira et al. 1998). A female betta may eavesdrop on two fighting males and then select the winner to be her mate. But how does a female choose if she is not given the opportunity to watch two males fight? How does she decide which male to pursue without wasting costly energy engaging in courtship rituals with both? To fully comprehend the factors contributing to female mate selection, the differences between male and female betta must be further explored.

Betta are a sexually dimorphic species and thus exhibit differences in appearance and size between sexes. Sexual dimorphism can refer to physiological differences or size differences with many species exhibiting both. Some of the most drastic examples of sexual dimorphism are seen in birds and fish (Lande 1980). In many species, such as betta, the males are larger than the females due to the frequency of male-male combat. This size difference is an example of intrasexual selection as larger males tend to win more fights and are thus exposed to more females (Shine 1989). On average, male betta are approximately 30% larger than females but the two size ranges overlap, causing some females to be larger than some males (Clotfelter et al. 2006). In many species of fish, female size is directly related to fecundity so the larger the female, the larger the potential brood (Parker 1992; Shine 1998).

Sexual dimorphism can occur in a number of traits and for a number of reasons.
In some cases, the sex providing parental care to the offspring is generally less conspicuous in color to not attract attention to vulnerable offspring (Stevens & Merilaita 2011). Other times the choosy sex may select for a trait such as coloration or fin length. In several species of fish, such as the guppy (*Poecilia reticulata*) and betta, the females select males with longer fins and/or tails over males with shorter fins and/or tails (Allen & Nicoletto 1997; Nicoletto 1991). As a result of this sexual selection, the males look far different than the females in terms of fin and tail length. Betta females may prefer longer, undamaged fins because they indicate that the male is an excellent fighter and in good health (Allen & Nicoletto 1997). Yet longer fins can be costly to the organism thus there is a limit to the elaboration of this trait.

Fisherian runaway is the evolution of exaggerated male physiological and morphological traits as a result of sexual selection when the traits are not directly indicative of male fitness (Pomiankowski & Iwasa 1998). This phenomenon is a consequence of female choice when females of the species select for increasingly dramatic traits and the female preference in turn becomes more exaggerated. After a certain period of time, the exaggerated characteristics can become a cost to the individuals. The best-known and one of most exaggerated examples is the male peacock, whose costly tail is far more of a burden than it is of use (Petrie et al. 1991; Zahavi 1975). In addition to having long tail feathers that make flight impossible, the males are also brightly colored making camouflage all but impossible (Burgess 2001; Fisher 1958). Wild male betta do not have the same fin length as commercially bred fish because fin length in the wild is costly to the individual. Larger males that win more fights and longer fins attract more females (Allen 1997), but being too large and having excessively long fins becomes costly when fighting other males. These males would most likely not survive in the wild long enough to mate (Wallbrunn 1958). This tradeoff occurs in most
sexually dimorphic species where a desirable trait is selected for. This is one way for a female betta to select potential mates, but there are a number of other factors that also play a role.

Non-desirable characteristics such as outward signs of parasitic infection can also influence a female’s mate choice. Several scientists including Hamilton and Zuk (1982) and Smith (1985) argue that parasite-mediated sexual selection can be a powerful evolutionary force because the rapid evolution of parasites leads to sustained fitness benefits of choosing parasite-free males. Thus, it is important for a female to be able to determine the male's resistance to parasites so her offspring have the best chance of also being parasite-resistant, given that immunocompetence can be heritable (Smith 1985). In betta, a female can assess immunocompetence in several ways. First, if she engages in courtship with the male and he lacks vigor this could indicate that he is infected with parasites or other pathogens and is overall not immunocompetent. Lethargy could be indicative of other things, such as the male being a poor forager, which also would also not be desirable. In addition to lethargy, color is an important factor in female mate selection. Bright coloration in a male indicates that he is a successful forager given that he has consumed enough carotenoids to support his immune system and coloration (Blount et al. 2003). Bright coloration also indicates that he is healthy and free of pathogens as infected organisms tend to be duller in color (Faivre et al. 2003; Houde & Torio 1992). In addition, the fins are also an important visual indicator for health. When betta are immunocompetent and successful foragers, their fins tend to heal from minor injuries within one to two days (Castro et al. 2006). If a male has many marks along his fins that are not healing or are infected, the female is likely to not pursue him. The female will take note of all of these factors during the beginning stages of her courtship with the male.
Finally, genetic compatibility may be a subconscious factor in mate selection (Tregenza & Wedell 2000). In some species, individuals prefer mates that carry dissimilar genes at the major histocompatibility complex (MHC). It is believed that this is linked to odor and evolved due to benefits of increased MHC heterozygosity in the offspring (Penn 2002). Odor works well for organisms such as mice, primates, dogs, and other land dwelling mammals, but fish often require a different way to detect genetic compatibility (Klein 1986; Penn 2000). A study on *Pseudotropheus zebra* showed that fish initially preferred allopatric color variants when given an option, suggesting that color indicates genetic differences in this species (Knight & Turner 2004). In cichlids, color plays a role in mate selection, but it is hypothesized that sound may also play a role (Amorim et al. 2004). Alternatively, some kinds of olfactory cues can suggest compatibility in swordtails and sticklebacks (Malinski 2003; McLennan & Ryan 1997; McLennan 2004). In other species, sound is produced during courtship, but female mate selection is based largely on olfactory cues (Plenderleith et al. 2005). Currently it is not known how betta determine genetic compatibility. A study looking at betta aggression suggested that visual cues resulted in a more aggressive response than olfactory cues (Lissmann 1932). Regardless of the sensory information, all of the above mentioned sensory cues play a role in betta mating behavior.

**Courtship Behavior**

Betta fish are able to breed year around because they do not have a specific biological breeding clock. They tend to breed successfully in conditions where the temperature is close to 26.7°C as they are native to warmer climates (Bronstein 1981b). They prefer still or very slow-moving water, such as canals or rice fields, with low vegetation or floating leaf litter (Raja & Sampath 2004). In addition, betta tend to prefer low-light environments as opposed to direct sunlight. When these conditions are met,
male betta construct isolated or low traffic territories where they are unlikely to be bothered. These territories are generally rather small and may border other male's territories, but are protected fiercely (Braddock & Braddock 1955). The males become temporarily attached to their territory and are unlikely to leave once they begin constructing bubble nests (Bronstein 1981a).

The display of *Betta splendens* is unique in that the behavior witnessed in intraspecific interactions and territory disputes is very similar to what is witnessed during courtship behavior. During the breeding season, males fight over and defend territories where they have built nests and attempt to court females (Bronstein 1981a). Whereas decisions over mating interactions may seem to be made by both sexes, female betta are actually more selective than males. The female chooses a male based on two overarching factors; genetic quality and resource holding potential (Clotfelter et al. 2006).

First and foremost, the male must be genetically compatible in order to increase genetic diversity of the offspring (Wallbrunn 1958). Resource holding potential is also extremely important because the males must be able to care for and protect the offspring until they are able to leave the nest (Rainwater & Miller 1967). The female determines this based on secondary characteristics, such as coloration, flashiness, vigor of the display, and evidence of parasites, in addition to examining the male’s territory and bubble nest (Clotfelter et al. 2006). Males are less choosy because the females provide little to no parental care (Simpson 1968). Female size is not a predictor of fecundity so there are few visual cues other than evidence for parasite infection for the males to select for or against (Clotfelter et al. 2006). With these things in mind, the specific courtship behavior of *Betta splendens* can be examined. The behavior is generally broken up into two different categories; prespawning and postspawning behavior (Rainwater & Miller 1966). Prior to a mating encounter, however, the male must construct a bubble nest to
Male betta construct bubble nests by taking a bubble of air into their mouths, enclosing it in mucus, and depositing it on the surface of the water. They do this repeatedly until layers of bubbles are evident, forming a floating white mass on the surface of the water (Braddock & Braddock 1955). The nest location and size depend primarily on environmental factors and genetic influence (Jaroensutasinee & Jaroensutasinee 2001). In general, males prefer to nest under a broad leaf or something floating on the surface of the water. If a leaf is not available, fish in aquariums will nest along the edges of the tank (Braddock & Braddock 1955). The bubble nest is important because it serves as the nest for the fertilized eggs and recently hatched offspring (Braddock & Braddock 1955). For this reason, females will mate only in the presence of a nest and they inspect it prior to mating. Males aggressively chase females away if their nest is not complete or missing entirely (Rainwater & Miller 1967). There are varying reports about the importance of size and the shape of bubble nests to females. It is known that nest size depends on the size of the male, but nest size is not correlated with the number of breeding opportunities (Clotfelter et al. 2006). There are also varying reports about the consistency of nest shape among males and whether or not that plays a role. Some say that shape is consistent as long as environmental conditions do not dramatically change (Jaroensutasinee & Jaroensutasinee 2001). Others report that nest size varies dramatically between males and even for the same male (Braddock & Braddock 1955). Nevertheless, upon completion of a bubble nest the male will be ready to court a receptive female.

While males will not mate unless a bubble nest is present, the female in turn will mate only if she has mature ova as evident by the extending gravid tube between her ventral and anal fins (Clotfelter et al. 2006). Body coloration is also an indication of each
sex being ready to mate. Males become brighter, flashier, and more brilliant in coloration. The females exhibit darker, horizontal stripes along the length of their bodies with lighter stripes in between (Rainwater & Miller 1966). Both of these characteristics indicate that the fish is healthy, a good forager, and parasite free. The colors are apparent from a distance and increase in intensity only as long as the pair continues to interact. An approaching female views these characteristics and makes a decision as to whether or not to approach the male.

When the female comes within roughly 12 cm of the male, they begin to interact (Personal Observation). The pectoral fins, which are located along the belly directly beneath the gills, and most of the dorsal fins, which are located along the top of his back, become be erect and tense or “flared” in both males and females. Soon after or in conjunction with, the gill covers become erect (or “flared”), making the male’s face appear larger (Simpson 1968). This courtship display appears identical to the aggressive display the male exhibits when a rival male enters his territory. In response to the male, the female also flares her pectoral and dorsal fins, which are generally smaller than males’ fins. Both male and female may now swim in rapid, darting motions towards each other while nipping at each other’s fins, with the male primarily doing the chasing (Rainwater & Miller 1967). Any physical encounters are very brief at this stage and serve only to show off to the opposite sex. More commonly, the male zigzags away from the female to show coloration, fin length, and body size. Water undulations under the fins cause the scales to shimmer and the color to appear more vibrant, which attracts females (Simpson 1968). From here, the male either continues to chase and nip at the female, or he will lead her to the nest.

If both male and female are receptive, the male leads the female to his nest in the same zigzag motion he used prior to showing his coloration and fins. Upon reaching the
nest, he will present it to her by stopping beneath, positioning his body upwards, and undulating his body (Simpson 1968). From there, he will intermittently work on his nest and display to the female to encourage her to come closer (Rainwater and Miller, 1966). At this point, the male must not be too aggressive or too passive. Too aggressive or vigorous of a display might drive the female away, yet too passive of a display might cause her to lose interest (Clotfelter et al. 2007). This portion of the prespawning stage either takes place relatively quickly or may require several rounds of chasing, courting, leading, and showing the bubble nest before the female approaches (Rainwater & Miller 1966). When the female approaches to inspect the nest, the male ceases nest building and folds his dorsal fin. Her approach is slow and deliberate, unlike the rapid bursts of movement seen previously (Simpson 1968). She inspects the nest for thickness and stability, among other things, to determine if it is fit for her offspring (Jaroensutasinee & Jaroensutasinee 2001). At this point, she may decide to reject the male or the pair will begin the next part of the courtship sequence.

At this stage, the male tightly circles the female beneath the nest. The male moves fairly rapidly, periodically stopping to flare his fins, prior to continuing. The female may follow the male or circle in return, but generally the male does most of the displaying (Rainwater & Miller 1966). The duration of the circling behavior is generally brief compared to the other portions of the prespawning stage, but it varies from pair to pair. This behavior is generally preceded by the mating pair's first physical contact, or their first clasp. The male folds his body in a U shaped position with the head and caudal fins pointed downwards (Simpson 1968). The female is positioned between the male’s head and caudal fins in a sigmoid position, rolled so she is upside down. The pair performs several preliminary clasps to practice orientating their bodies and synchronizing their movement (Rainwater & Miller 1966). Once the pair is ready and comfortable, they will
move on to the spawning stage.

At this point, the mating pair clasp again with the female upside-down, and the male draped over her body. They are positioned close to the surface of the water either directly below the bubble nest or right next to it. The male then tightens his clasp to stimulate ovulation, which occurs almost immediately concurrent with ejaculation (Rainwater & Miller 1966). The pair slowly sink during egg release and ejaculation so this encounter is short. The pair will often remain motionless for several seconds to recover before the male begins egg recovery. The male is positioned so he is facing downwards so he can see the eggs fall. After the female releases the eggs, the male catches them in his mouth before they reach the ground, carries them to the bubble nest, and injects them into specific locations (Simpson 1968). The female is often observed helping, but it is the male who catches most of the eggs. It is important for the majority of the eggs to be retrieved before reaching the bottom, where they are far more difficult to find and retrieve (Jaroensutasinee & Jaroensutasinee 2001). The pair repeats this step several times until the female departs and the mating ritual is complete.

Through understanding some of the broad cues male and female betta use to select mates, specific cues can be examined. By examining select, specific cues, portions of the complex hierarchy of sexual characteristics and their relation to mate selection can be better understood. The goal of this thesis was to understand how females select a mate when some olfactory and visual cues such as size, aggressiveness, and fin length were controlled for. The two cues of interest, coloration and movement, will be investigated in this next chapter.
Chapter Two

Female mating preferences as a result of coloration and movement in Betta splendens

Introduction

Sexual selection, as introduced by Charles Darwin, is an important evolutionary agent as it explains the existence of traits, how they are selected and favored, and the variability of traits among organisms (Darwin 1859). Females are often the choosy sex when faced with multiple prospective mates, and base their decisions on secondary sexual characteristics such as body size, coloration, ornamentation, and vocalizations (Arrow 1951; Fisher 1930; O'Donald 1962). These secondary characteristics may have arisen as the products of three proposed mechanisms: (1) direct fitness benefits such as parental care or enhanced fertility (Fisher 1930), (2) indirect genetic benefits such as inheritable genes for viability (i.e. parasite resistance, offspring fitness) (Flor 1971), and (3) the result of preexisting sensory biases such as vocalizations (Ryan & Rand 1993). It has been hypothesized that females benefit from being selective through direct, material benefits as they are obtained in the present generation (Moller & Jennions 2001). Indirect benefits are alternatively obtained in the subsequent generation and do not immediately impact the choosing female (Kotiaho & Puurtinen 2007).

Both sexes respond innately to mating opportunity costs regardless of which sex devotes the most effort to parental care. While females are generally more restricted in the number of times they can mate and often provide the most parental care, males must also be selective of perspective females (Gross & Sargent 1985; Moller et al. 1998). So how do females find and select an appropriate mate from the population without wasting
unnecessary energy, time, and resources (Janetos 1980)? It is known that different cues have evolved to suggest the genetic capabilities (fitness) of males and females alike. These fitness related traits are theoretically maintained through two potential systems; one where females choose males with superior genes or a second system where females choose males who are genetically compatible (Hamilton & Zuk 1982; Tregenza & Wedell 2000). Genetic compatibility has been studied in mammals such as humans, mice, primates, and also in some fish, and it has been found that these species select mates with dissimilar genes at the major histocompatibility complex (MHC) based on olfactory cues (Ober et al. 1997; Wedekind et al. 1995; Yamazaki et al. 1988).

Sexual displays and courtship behavior are highly complex and species specific. Traits, displays, and courtship behaviors are often complex because they represent a rich evolutionary history that has been modified as the environment and population has changed over time (Buss 1989). Recent studies have shed light on the complexity of mate selection based on this context. For this reason, mate selection is often complicated with either sex selecting mates based on multiple traits and cues (Kleiman & Eisenberg 1973; Wcislo 1989). Depending on the context and the quality of the cue, one may be more important to the choosy sex. For example, in many species of birds, mates are selected based on bright ornamentation in addition to their ability to perform elaborate songs (phenotypic traits) (Badyaev et al. 2002; Garamszegi 2004; Garamszegi 2005). The choosy sex will distinguish between mates by these sensory cues prior to expending costly energy engaging in direct behavior. Multiple cues may do any of the following: (1) increase fitness by reducing errors in mate choice or the costs of choosing a mate (Candolin 2005; Dale & Slagsvold 1996); (2) consist of remnants from past selection and/or traits that arose separately from mate choice, while having no current influence on fitness (Wells et al. 2009); (3) may actually decrease fitness but is still selected for due to
preexisting sensory biases (i.e. runaway selection hypothesis) (Candolin 2003; Pomiankowski & Iwasa 1998). With this in mind, cues can further be divided into two categories; informative and uninformative. Informative cues indicate something about the fitness of the individual and can reduce mate choice costs and errors (Clotfelter et al. 2007; Jaroensutasinee & Jaroensutasinee 2001). Uninformative cues are not reliable indicators of mate quality and often involve a preexisting sensory bias to which the choosy sex is oblivious (Candolin 2002).

Many species of fish select mates based on multiple cues that either increase fitness or are remnants of past selection. For example, male European bitterlings (*Rhodeus sericeus*) attract females based on the presentation of a mussel as a resource for the offspring, and the coloration and courtship behavior of the male. Initially the female is attracted based on the male's coloration and courtship, but then the female's final decision is made based on the actual mussel quality (Candolin & Reynolds 2001). Similarly, in Mexican pupfish (*Cyprinodon* spp.) the different species discriminate among themselves during mate selection based on visual and chemical cues (Kodric-Brown & Strecker 2001; Strecker & Kodric-Brown 1999). This reduces the chance of error and prevents costly encounters that not result in mating opportunities.

*Betta splendens* is an excellent model species for studying the visual and behavioral interactions between male and female mate choice. First, they are a sexually dimorphic species with males being larger than females and having brighter colors and longer fins (Clotfelter et al. 2007). Second, both sexes are choosy with males competing for territories and partaking in the majority of the parental care, and females selecting males based on a variety of cues (ie larger size, aggression, etc.) (Clotfelter et al. 2007; Simpson 1968). *B. splendens* select mates based on multiple cues, many of which are based on phenotypic traits such as coloration, fin length, body size, and aggression. Other
cues involve offering a resource to the choosing female, such as a territory and bubble nest. It is known, for example, that female *B. splendens* prefer males who are aggressive to an extent, however, after a certain point, extreme aggression is no longer desirable (Lynn et al. 2007). It is also known that female betta prefer brightly colored males compared to dull males and examine the fin quality of the male (Castro et al. 2006). Both of these would indicate that the male is a good forager and is not immunocompromised, which in turn indicate good genes to pass to offspring. In addition, male betta provide parental care for the offspring, so avoiding a visibly parasite infested male could be directly beneficial to health of offspring. Despite knowing some of the cues that female betta select for, not much is known about the priority of these cues, or what matters more to the female.

The purpose of this study was to determine the relationship between two different potential mate choice cues, coloration and courtship vigor, when direct contact is denied in domestic stock of *B. splendens*. First, I hypothesize that red coloration may be a cue for good genes, indicating the uptake of carotenoids through successful foraging (Clotfelter et al., 2007). If so, then female betta should spend more time engaging in behavior with red males than blue males if simultaneously offered both. Second, I hypothesized that courtship vigor is also a cue, indicating that the betta is healthy and a good forager. If this hypothesis is valid, then females will prefer males who are more vigorous than those who are less vigorous. From there, I also examined which cue is more important to a female when selecting a mate: courtship behavior or coloration. To accomplish this, I conducted four experiments to determine the relationship between these two potential mate cues. In Experiment I, the roles of coloration and vigor were independently examined to investigate the two as being mate cues. In Experiment II, males were anesthetized using MS-222 to determine if there were any short and long term
effects on behavior. In Experiment III, male vigor was manipulated using anesthetic to determine the relationship between coloration and vigor. In Experiment IV, cutout models of male betta were offered to females in place of live males to further examine the relationship between coloration and vigor.

**Experiment I**

In this experiment I investigated male coloration as a possible mate cue by simultaneously offering both red and blue colored males to female betta. Behavioral data from this experiment also allowed me to examine the relationships between male and female vigor.

**Materials and Methods I:**

I obtained 10 male and 10 female bettas between the months of November 2013 and July 2014 from two local pet stores in Gurnee, IL and Highland Park, IL. Five of the males were predominantly red colored and five of the males were predominately blue colored. The females varied in coloration. The age of the fish was not known and it was unknown if the females were virgins. The fish were kept individually in 8 liter plastic tanks containing neutral colored gravel and filled with filtered and treated water. Paper was placed around each tank to prevent the fish from seeing each other. Each male was housed in its individual aquarium for a minimum of one week prior to testing. Each female was housed in its individual aquarium for a minimum of three weeks prior to testing. The room temperature was kept between 24.5°C and 27.8°C. The betta were fed a color enhancing food (Aqueon Betta Color Enhancing Pellets) once daily and kept on a 12:12 light/dark schedule. During the data collecting weeks, the fish were fed twice daily: once prior to testing and once at the end of the day after testing.

Trials were conducted at various times between the hours of 7:00 am and 4:00
pm. Immediately after testing the fish were anesthetized and photographed to obtain measurements. Standard length was measured as the distance between the mouth and base of the caudal fin. Tail length was measured as the distance between the base of the caudal fin to the end of the longest fin ray. Total body length was then calculated as the sum of standard length and tail length.

To test the hypothesis that female betta spend more time with and are more vigorous towards red colored males, I conducted trials in which one male of each color was simultaneously offered to the female. Trials took place in one of two ten gallon (50.8cm x 27.9cm x 33.0cm) tanks containing neutral colored gravel and filled with approximately 20cm of filtered and treated water (Figure 2). Black paper was taped to three sides of the tank to visually isolate the fish being tested. The tank was divided into three equal zones (16.9cm wide) to create a male zone, a center neutral zone, and another male zone. The room was dimly lit and quiet during trials.

Figure 2. Apparatus for Experiment I. The tank is divided into three equal zones (male-neutral-male) with each color male being placed in the beakers.
There were 10 males and 10 females used in this experiment. Each female was tested ten times with different pairs of males. Each female was tested a maximum of one trial per day, five times per week with two consecutive days off between testing weeks. The males were used a maximum of three times per day. Each male could be used two consecutive times with a minimum of 20 minutes off between trials. If used a third time, the male was placed back in its tank for a minimum of two hours before being used again. All males were tested a maximum of five days a week with two consecutive days off between testing weeks.

For each trial, the female was placed in the experimental tank for 20 minutes of isolated acclimation time. After roughly 15 minutes, the red and blue colored males for the trial were placed in a separate 800 mL glass beakers containing approximately 500 mL of filtered and treated water. The males were then carried near the experimental tank and allowed to acclimate for several minutes in view of each other, but out of the view of the female. The female was then gently moved to the neutral zone of the tank using a small fish net. The beakers containing the males were placed in the center of each male zone at the ends of the aquarium. The beakers were not fully submerged so the male betta remained isolated inside the beakers. The timer was started and the experiment began. I recorded the following during the trial; total time the female spent engaged in any behavior (i.e. the female interacted in any way with the male) with each male, female and male vigor, female frequency of each behavior, and the behavior of the male. I recorded the following behavior of the two males and the female during the trial period: the time spent looking, flaring, circling, and/or quivering (Table 1). Male and female vigor was scored based on the behavior witnessed during trials (Table 2).
<table>
<thead>
<tr>
<th>Look</th>
<th>Within 6 cm and facing the other fish. Either swimming towards the other fish or non-moving.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flare</td>
<td>Erect operculum, pectoral fins, and/or dorsal fins. Either swimming towards the other fish or nonmoving.</td>
</tr>
<tr>
<td>Circle</td>
<td>Swimming either consistently or in short bursts around the other fish. Periodically flare.</td>
</tr>
<tr>
<td>Quiver</td>
<td>Female: Non-moving female rolls slightly to side while vibrating body rapidly. Male: Same as female except occasionally folds into U-shaped position.</td>
</tr>
</tbody>
</table>

Table 1: The categorized behavior witnessed during trials by male and female betta.

<table>
<thead>
<tr>
<th></th>
<th>Not Moving</th>
<th>No movement or behavior towards other fish. Limp/unmoving.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Low Vigor</td>
<td>Occasionally looked and flared at other fish. Some movement and interest.</td>
</tr>
<tr>
<td>2</td>
<td>Vigor</td>
<td>Frequently looked and flared at the other fish. Reciprocated some of the other fish's movement.</td>
</tr>
<tr>
<td>3</td>
<td>High Vigor</td>
<td>Always looked and flared when other fish was near. Frequently reciprocated the other fish's movement.</td>
</tr>
<tr>
<td>4</td>
<td>Very High Vigor</td>
<td>Same as Very Vigorous. Quivered at least once during the trial.</td>
</tr>
</tbody>
</table>
Table 2. The numerical values and behavior observed for the different levels of vigor.

**Results**

When female betta were given a choice between red or blue colored males, females preferred (spent more time with) red colored males in 118 of the trials compared to preferring blue colored males in 60 of the trials (Figure 3. Binomial test: $p < 0.001$). Female betta also spent significantly more time engaged in behavior with red colored males than they did with blue colored males (Figure 4: $t = -4.78$, df = 177; $p < 0.001$). When looking at individual females and their overall preferred color, 10 out of 10 females preferred red colored males compared to blue colored males (binomial; $p = 0.002$).

![Bar chart showing preference between red and blue males](chart.png)

Figure 3. Female *Betta splendens* preferred red colored males over blue colored males in significantly more trials than the reverse.
The vigor of female behavior increased as the behavior of each male increased. (blue male - female: $r_{98} = 0.354; p < 0.001$; red male - female: $r_{98} = 0.612; p < 0.001$). As blue male vigor increased, red male vigor also increased (blue-red: $r_{96} = 0.473; p < 0.001$). This suggests that female and male behavior were correlated.

**Experiment II**

The goal of this experiment was to reduce male levels of vigor using MS-222 in addition to determining the short and long term effects of daily anesthetization.

**Materials and Methods II**

I obtained 15 new females in July 2014 from two local pet stores. Ten solid colored red males and ten solid colored blue males were selected from previous experiments based on consistent, high levels of vigor and behavior. The females and males were both given two weeks to acclimate or recover from previous experiments.
The females varied in coloration. The age of the fish was not known and it was unknown if the females were virgins. The fish were kept in the same conditions and feeding schedule as Experiment I.

MS-222 (250 mg/1 L) was used to immobilize male betta, in effect reducing their level of vigor to zero. In order to ensure the effectiveness of using MS-222 and to determine if there were any subsequent effects on behavior, I observed the behavior of males daily before, during, and after anesthetization for a total of five days. Trials took place in a 22.8 cm x 15.2 cm x 16.5 cm plastic tank containing neutral colored gravel and filled with approximately 14 cm of the same filtered and treated water. Black paper was taped to three sides of the tank to visually isolate the fish being tested.

There were 6 females and 6 males (3 red and 3 blue) used in this preliminary experiment. They were arbitrarily selected from the previous 18 females and 20 males used and had two weeks of no testing since Experiment I. Each female was used once per day for five consecutive days. Each male was also used once per day for five consecutive days. Each trial consisted of three stages: the first stage where the male was not anesthetized, a second stage where the male was anesthetized, and a third stage 24 hours later where males were not anesthetized.

For each trial, the female was placed in the experimental tank for 20 minutes of isolated acclimation time. After roughly 15 minutes, the male was placed in an 800 mL glass beaker containing approximately 500 mL of filtered and treated water. The male was then allowed to acclimate near, but visually isolated from the experimental tank for several minutes. The beaker containing the male was then placed in the center of the tank, regardless of the female’s location (Figure 5). The timer was started and the experiment ran for five minutes. The following was recorded during the trial: total time the male spent engaged in any behavior with the female, as well as female and male vigor.
Figure 5. Apparatus for experiment two. Each male was placed inside the beaker whereas the female was placed inside the tank.

The male was then removed and placed in an 800mL beaker containing 500mL of MS-222 solution (250mg/L). When the male was fully anesthetized (limp on the bottom of the beaker), he was carefully propped upright with a clear, glass stir rod and placed back in the center of the experimental tank containing the female. This second trial lasted for five minutes. The male was placed in a small recovery tank for 30 minutes and then transferred back to its individual tank for 24 hours.

**Results II**

Each male was anesthetized daily for five days to determine the short/long term effects on behavior and vigor in females. MS-222 successfully anesthetized the males and significantly reduced the amount of time engaged in behavior (Figure 6: t = 30.599; df = 29; p < 0.001). After 24 hours of recovery, the amount of time spent engaged in behavior increased compared to when anesthetized (t = -43.312; df = 23; p < 0.001). The males returned to pre-anesthetization levels of vigor within the 24 hours of recovery time (t = 1.218; df = 23; p = 0.236). This showed that there were no short-term effects on male
behavior due to anesthetization.

Figure 6. The time male B. splendens spent engaged in behavior awake, anesthetized, and then awake again for all five days. Error bars represent a 95% confidence interval.
Figure 7. The amount of time each male spent engaged in behavior pre-anesthetization during each of the five days of testing. The lines represent each individual male. B6, B8, and B8 are blue-colored males while R7, R8, and R10 are red-colored males.

During the five days of testing, there was no significant decrease in the time that males spent engaged in behavior pre-anesthetization (Table 3). The generally low $R^2$ values indicate that there was no regressive relationship between the day and the time as a result of anesthetization using MS-222. This indicates that daily anesthetization did not negatively impact the long-term behavior of the male. The only male that had a higher $R^2$ value was B8 ($R^2 = 0.8623$). It is known that MS-222 stresses fish in different ways (Thomas & Robertson 1991; Wedemeyer 1970) but the exact impacts on *B. splendens* are unknown. For this reason, B8 was not used for future experiments and males were anesthetized on only two consecutive days and for a maximum of three times a week.
<table>
<thead>
<tr>
<th>Male</th>
<th>$R^2$</th>
<th>Slope</th>
<th>Average Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B8</td>
<td>0.8623</td>
<td>-14.4</td>
<td>226.8</td>
</tr>
<tr>
<td>B6</td>
<td>0.0354</td>
<td>-1.6</td>
<td>285.6</td>
</tr>
<tr>
<td>B9</td>
<td>0.3972</td>
<td>-9.8</td>
<td>262</td>
</tr>
<tr>
<td>R7</td>
<td>0.2368</td>
<td>-1.8</td>
<td>281.2</td>
</tr>
<tr>
<td>R8</td>
<td>0.0521</td>
<td>-5.3</td>
<td>254</td>
</tr>
<tr>
<td>R10</td>
<td>0.0448</td>
<td>1.9</td>
<td>268</td>
</tr>
</tbody>
</table>

Table 3. $R^2$ values and slopes for the time spent engaged in behavior pre-anesthetization for each of the males used in experiment two (Figure 7).

**Materials and Methods III**

To examine the relationship between courtship behavior and coloration in mate choice, I ran trials in which females were presented with a red and a blue male, in all combinations such that the red male was either anesthetized (not moving) or unanesthetized (moving) and the blue male was either anesthetized (not moving) or unanesthetized.(moving). Trials occurred in the same ten gallon tanks that had been used in experiment I with the following changes: the water level was raised to 20 cm and two 100mL beakers were placed in the center of both male zones (Figure 8). There were 15 new females used and 16 males (8 red and 8 blue) selected based on consistent, high levels of vigor during previous experiments. The males received a minimum of two weeks off of testing prior to being used in this experiment, while the females received three weeks to acclimate.
Figure 8. The experimental set up for experiment three. The two larger beakers were placed on top of two smaller beakers to allow for greater visual contact by the female. The two males were placed inside of the larger beakers.

Females were used a maximum of one time per day for no more than five consecutive days before receiving a minimum two consecutive day break. Males were used a maximum of two times per day and were anesthetized up to once per day for a maximum of three days per week. The males also received a minimum two consecutive day break between testing weeks. Each trial involved one female and two males.

For each trial, the female was placed in the experimental tank for 20 minutes of acclimation time. Each trial lasted for ten minutes. Each female underwent four trials involving the four combinations of moving and non-moving males. Every female was offered completely different pairs of males during each of the four different trials. The following four conditions were tested in arbitrary order: Both Move, Red Move, Blue Move, and No Move. In both move, the test mimicked experiment one in that both males were unaltered. In red move trials, the blue male was anesthetized and the red unaltered. In blue move trials, the red male was anesthetized and the blue unaltered. In no move
trials, both the red and blue males were anesthetized.

For each trial, the female was placed in the experimental tank for 20 minutes of isolated acclimation time. After roughly 15 minutes, the red and blue males were obtained. Males that were not to be anesthetized were placed in an 800mL glass beaker containing approximately 500 mL of filtered and treated water. Males that were to be anesthetized were placed in an 800 mL glass beaker containing a MS-222 solution (250 mg/ 1 L). When the male was fully anesthetized (limp at the bottom of the beaker), he was carefully propped upright with a clear, glass stir rod. The female was gently maneuvered into the neutral zone and both beakers were placed on top of the 100 mL beakers in the experimental tank. The timer was started and the experiment ran for ten minutes. The following was recorded during the trial: total time the female spent engaged in any behavior with each male, female and male vigor, female frequency of each behavior, and the behavior of the male. Anesthetized males were then moved into a recovery container for approximately 30 minutes.

Results III

A One-way Analysis of Variance was used to analyze the difference between the preference ratios ((R_time-B_time)/(R_time+B_time)) to determine if movement was an important cue for female betta. There was a significant difference of mean preference ratios between trial conditions (F_{3.56} = 27.318; p < 0.001). Compared to trials in which both males were not anesthetized, females showed significantly altered preference when red males were anesthetized, switching their preference to blue males (Tukey HSD; p < 0.001). However when blue males were anesthetized, the females’ preference for red males was not changed significantly (Tukey HSD; p = 0.134) (Figure 9).
Figure 9. A ratio $((R_{time} - B_{time})/(R_{time} + B_{time}))$ of the time the female spent engaged with the red colored males minus the blue colored males. Positive values represent a red preference and negative values represent a blue preference.

To determine if changes in female preference between trial conditions might be due to a change in overall degree of interest in males, an additional One-Way Analysis of Variance test was conducted on the total time $(R_{time} + B_{time})$ the female spent engaged in behavior with either male. There was a significant difference in total engagement time among trial conditions ($F_{3,56} = 8.569, p < 0.001$). Females did not spend significantly more time engaged in behavior when red males were not anesthetized compared with total time when both males were not anesthetized (Tukey HSD; $p = 0.622$) (Figure 10). However, there is a significant difference in the total time the female spent engaged in behavior between trials where both males moved and trials where only blue males moved (Tukey HSD; $p = 0.0217$; paired t-test; $t = 5.233; df = 14; p < 0.001$). This indicates that the time spent with males was dependent on the courtship behavior of the red male.
Materials and Methods IV

In this experiment, females were tested against paper cut outs in a class project to examine the relationship between color and movement as cues in mate choice. Seven females of various color were obtained and housed in one liter plastic tanks containing only filtered and treated water. The female's ages were not known and it was unknown if the females were virgins or not. Paper was placed around each tank to prevent the fish from seeing each other. They were housed separately from the other females but were on the same 12:12 light/dark schedule and received the same food, once per day.

During trials, the female's tank was placed between two identical, completely empty tanks with paper obscuring the view (Figure 11). Two paper cutouts were made: one cutout resembled a red male betta and another resembled a blue male betta. The shape and size of the male cutouts were identical. Three timers were obtained: one timer
was used to count down the trial time (two minutes per trial), another was used to keep track of the time when the female was observed actively fixated on the red male, and another was used to keep track of the time when the female was observed actively fixated on the blue male. The female was determined to be actively fixated on a male if she swam against the plastic separating the two tanks. At the start of each trial the papers separating the tanks were removed and the timer was started.

Trials for four different types of conditions were carried out: in the first condition neither cutout was moved, in the second the red cutout was moved mimicking male movement, in the third the blue cutout was moved, and in the fourth both cutouts were moved. The female betta were tested in random order and the trial condition was randomly selected. Females were given at least one hour of rest before being tested again and were tested a maximum of two times per day.

![Figure 11. Apparatus for experiment IV. The female was placed in the middle tank and the male cutouts were used in the two adjacent tanks.](image)

**Results IV**

A One-way Analysis of Variance was used to analyze the difference between the preference ratios \((R_{time}-B_{time})/(R_{time}+B_{time})\) in male cutout models. There was a nearly significant difference of mean ratios between trial conditions \((F_{3,20} = 2.892; p = 0.061)\).
When a paired t-test was done to analyze the difference between ratios when only red cutout models move and when both cutout models move, there was not a significant effect of a nonmoving blue cutout model ($t = -0.196; \text{df} = 5; \text{p} = 0.852$) and the confidence interval did not include zero. However, comparison of the mean ratio from trials when only blue cutout models moved and trials when both cutout models moved (both move) was significant (paired t-test; $t = 2.573; \text{df} = 5; \text{p} = 0.050$) and the confidence interval did not include zero (Figure 12).

![Figure 12](image)

**Figure 12.** A ratio (($R_{\text{time}}-B_{\text{time}})/(R_{\text{time}}+B_{\text{time}})$) of the time the female spent engaged with the red colored males minus the blue colored males. Positive values represent a red preference and negative values represent a blue preference. Error bars represent 95% confidence interval.

When a One-Way Analysis of Variance was run on the total time the female spend engaged in behavior with either cut out, the difference between each trial condition was not significant ($F_{3,20} = 1.41; \text{p} = 0.269$) (Figure 13). This indicates that when using male cutout models, there was not much difference in interest due to movement.
Figure 13. The total mean time ($R_{\text{time}}$ plus $B_{\text{time}}$) the female spent engaged in behavior with either cutout.

Discussion

Coloration and vigor are both mate choice cues for female $B.\ splendens$. Female betta spent more time engaged in behavior with red colored males versus blue colored males when both were simultaneously offered. Female and male courtship vigor are correlated and female betta spend more time engaged in behavior with a vigorous male over a non-vigorous male, regardless of coloration.

The preference for red coloration may reflect the role of carotenoid dependent signaling. Carotenoid pigments are obtained through foraging and fulfill various physiological roles including influencing visual pigment function, epidermal growth hormone, and spectral filtering (Vershinin 1999), as well as enhancing immunocompetence (Clotfelter et al. 2007). In betta and guppies ($Poecilia reticulata$), the females prefer males with high levels of carotenoids. Carotenoid-based coloration is
speculated to indicate that in both betta and guppies the male is a successful forager and is not infected with parasites (Clotfelter et al. 2007; Grether 2000). In my study, carotenoid pigments were controlled through offering both colored males the same amount of a color enhancing food, which contained carotenoid supplementations. Clotfelter et al. (2007) further examined the role of carotenoid pigments and pteridines, an aromatic compound known to play a role in red pigmentation. Fish that were supplemented with carotenoid pigments developed higher carotenoid concentrations in their skin and became visually redder in color. This is true for many red or orange colored fish (Henze et al. 1977) and lizards (Macedonia et al. 2000).

The role of pigmentation in *B. splendens* mate choice has only recently been studied, but it is known that many species of fish show a preference for carotenoid-dependent signals (Candolin 1999; Kodric-Brown 1993; Maan et al. 2006). In betta, there is a trade-off between immunity versus coloration for carotenoid pigments. Whereas red-colored betta became redder in color when supplemented with carotenoids, blue-colored betta show no change in color. However, blue-colored betta mount an even greater inflammatory response when compared with red-colored betta (Clotfelter et al. 2007). This suggests that a brighter red coloration in males can not only serve as a secondary sexual characteristic to predict the health and foraging abilities of a the male, but can also predict the immunocompetence of the male. As there is no change in coloration in blue-colored males, females would be unable to determine immunocompetence and foraging abilities from coloration alone. The female betta would have to resort to other cues that indicate immunocompetence and foraging abilities, such as vigor.

When examining male and female vigor, I found that higher levels of male vigor, regardless of the color, were correlated with higher levels of female vigor. This suggests that there may be an additional factor in female betta mate selection and that color is not
the only secondary sexual characteristic that is taken into consideration. It is known that aggression (i.e. damaging nips to the fins, ramming, or excessive chasing) is important to a female betta as she should select a male aggressive and large enough to protect the offspring, yet aggressive behavior is not a runaway trait in the sense that an overly aggressive male presents a threat to females (Bronstein 1981b). Male betta are often larger than females and can cause severe fin or internal damage during mating encounters (Bronstein 1981b). The female gains information regarding male aggression generally through eavesdropping (Matos & McGregor 2002). In the absence of this opportunity for the female during my testing scenario, it is possible that aggression can be considered to be less important only because this information is not readily available to the female.

Vigor is potentially another factor that females consider when selecting between the red and blue-colored males.

Vigor was addressed in experiments three and four where males were anesthetized so their level of vigor was zero. When both males were not anesthetized, red-colored males were preferred. When blue males were anesthetized, females did not increase their preference level towards red-colored males when compared to total preference when both males were not anesthetized. However, when red males were anesthetized, blue males were preferred over red males. If red coloration was the only secondary sexual characteristic selected for by the female, then female betta would still prefer the non-moving red male over the moving blue male. The fact that preference was reversed indicates that vigor is an additional secondary sexual characteristic that the female will select for when choosing a mate. A male that is able to successfully forage (thus obtaining carotenoids), would have more energy and would be able to be more vigorous. Vigor is also indicative of a healthy male that is not heavily infected in parasites (immunocompetence) and is a good forager, exactly like red coloration. Vigor could then
be considered as both an alternative secondary characteristic to coloration and as a carotenoid-dependent signal.

Since female betta were denied the opportunity to eavesdrop on fighting males in this experiment, they had to engage in behavior with the male to assess vigor. This practice is costly as the female expends energy to engage in courtship behaviors with the male in addition to putting herself in danger of being injured or killed. Simply viewing the coloration of males puts the female in less danger and does not cost energy, which is why vigor is theoretically an alternative secondary sexual characteristic to coloration. However, in the wild, the female would be able to readily eavesdrop on fighting males and the findings of this study may not be representative for those settings. In the wild, females almost always select the winning male, regardless of physical appearance (Doutrelant & McGregor 2000). In my study, the males were in visual contact with each other during trials and may have adjusted their vigor as a result, but they were unable to physically contact each other. Without this physical contact between males, female betta would not be able to eavesdrop effectively and coloration would remain the primary deciding factor in mate choice.

MS-222 successfully anesthetized the males, because the fish were completely limp and unresponsive to any stimuli. A clear rod was therefore needed to gently maneuver the males to an upright position. While this made the male look more “normal”, there are limitations. The anesthetized male rested on the bottom of the beaker, a behavior not witnessed when fish were awake. Even during trials where males were not interested and had low vigor levels, the male was always observed floating somewhere in the beaker. Anesthetized males also became darker and duller in coloration. In trials where both males were anesthetized, the total time the female spent engaged in behavior was comparatively low. The cutout models were then used but had their own limitations.
The models were fairly rudimentary and the mimicked movement was not always an accurate representation of living specimens. It is uncertain how female betta viewed these models in comparison to live fish, even though they appeared to acknowledge them similarly to live betta. In the future, a more sophisticated approach could be done using a video presentation or digital model of a male that can mimic the desired behaviors. Several studies have already been conducted that show female betta do respond to digital models and have been used to determine preferences for aggression, fin length, and so on (Allen & Nicoletto 1997).

Our results show that female *B. splendens* select mates based on a variety of secondary sexual characteristics. Only through controlling for some of these characteristics is the relative importance of some of these cues distinguishable. By applying this approach to multiple, controlled situations, the relative hierarchy of characteristics can be distinguished, but in reality, it is highly situation dependent. The conditions of my experiment were drastically different than wild conditions, which may influence the results. By further studying carotenoid-dependent signals and secondary sexual characteristics a more complete understanding of betta mating behavior can be established.
References


