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Resource value as a mediating factor of aggression during within-sex competition between female Siamese fighting fish (*Betta splendens*)

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A departmental senior thesis submitted to the Department of Biology at Trinity University in partial fulfillment of the requirements for graduation with departmental honors.

April 16, 2015

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by

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Chapter One: A Review of Female Aggression

Just as any other phenotypic trait, aggressive behavior can be acted upon by selection, conferring fitness benefits upon individuals who allocate aggression in ways to reduce risks and enhance resource acquisition (Duckworth & Badyaev, 2007; Parmigiani et al, 1999). Although male aggression has been widely studied (Alcock, 1994; Le Boeuf, 1974; Meliska & Meliska, 1976; Wingfield, 1987), female aggression has received far less attention (Rosvall, 2011).

Female aggression is driven by competition for a variety of resources, from access to food and mate resources to protecting oneself and offspring (Arnott & Elwood, 2008; Cunningham & Birkhead, 1998). Female aggression is gaining attention as a topic of study because of the complexities driving this behavior. Many factors and selective pressures mediate female aggression, and as such it is important to integrate an understanding of social selection (competition for non-mate resources such as food) and sexual selection (competition for mate resources) into studies (Tobias et al, 2012). In this review chapter, I will be discussing the different selective pressures driving female aggression, hormonal causes of aggression, and conclude with some potential directions for future research.

Food is an important, finite resource that females often compete for. The strength of competition for these resources can vary upon availability and spacing; food resources can vary in both abundance and the distribution (Stamps, 1997). Female aggression has been shown to increase when food resources are low or hard to access and becomes stronger when females perceive that there is high competition for the few resources (Rosvall, 2011; Stamps, 1997). For example, in schooling guppies, there is a positive relationship between increased individual aggression and group size, which may arise due to increased competition for food resources (Magurran & Seghers, 1991). In another example, Stamps (1977) found that conflicts between

territorial female Bronze anoles increase with proportion of prey overlap. Another category of female aggression revolves around male interactions. Females face competition from males whose larger body size can prevent access to the highest quality food resources, and in an interesting study on Rufous hummingbirds, males were found to establish territories that contain denser flower resources, pushing out the smaller females into competition for lower quality territories (Kodric-Brown & Brown, 1978). In another example among American Redstarts, male aggression pushes females into competition for low quality wintering grounds and this negatively affects female health and body mass (Marra & Holmes, 2001). Changing energetic demands with regards to producing offspring can also impact female aggression. Females become more aggressive during pregnancy and lactation in mammals, which can aid in accessing food resources to fuel a higher metabolic demand (Rosvall, 2011). As such, an individual's energy demands, perceived competition, and ability to access food resources can all impact an individual's investment in aggression, and females have been shown to benefit from aggressive behaviors in all of these contexts.

Because female reproductive success is often influenced by mate quality and parental care provided by the male, it is widely assumed that females are the sex that most often exhibits mate choice (Cunningham & Birkhead, 1998). However, females are not passive choosers (Kempnaers, 1994). Competition for quality mates and paternal care can influence aggressive interactions between females. During the breeding season, many species of birds establish socially monogamous bonds, and pair-bonded males and females work together to defend a territory and raise offspring. In these cases, both males and females often defend their territory from intruding competitors (Fedy & Stutchbury, 2005; Breiehagen & Slagsvold, 1988), and females can take lead roles in defending a mate and the territory. For example, tropical female

birds lead duets with a mate during territory and mate defense (Langmore, 1998). Female aggression can even play an important role in determining a species' mating system. Female starlings enforce social monogamy through aggression towards other females (Sandell 1998), a pattern also seen in many other species of birds (Yasukawa & Searcy, 1982; Hau et al, 2004; Langmore, 1998). In many cases, primary females work to force out other females to ensure that their mate does not divide up paternal care competition for paternal care (Kempnaers, 1994), which sometimes forces the male into conflict with females to maintain stability between the females (Schradin & Lamprecht, 2000). In another example, juvenile plumage is used a signal of reduced competition in order to ward off aggressive encounters with adult females. Second-year female tree-swallows often retain their sub-adult plumage in order reduce aggression from adult females who would otherwise view an encroaching female as a competitor for her mate (Coady & Dawson, 2013). Female monopolization of paternal care, particularly in birds, can play a large role in the survivorship and quality of offspring (Wolf et al, 1988; Slagsvold & Lifjeld, 1994). It is thus favorable to a female to invest in aggression and monopolize access to her mate.

Females invest in aggression to protect offspring in a variety of contexts and in many taxa. Female aggression for offspring defense can be observed during pregnancy in mammals (Rosvall, 2011), where it has been found that lactation in rodent species stimulates aggression toward unfamiliar males entering the nest site (Erskine et al, 1998). In White's skink, a lizard, female aggression during pregnancy and the postpartum period results in higher survivorship of offspring (Sinn et al, 2008). Female dark-eyed juncos show more aggression at the nest towards same-sex intruders than opposite-sex intruders, perceiving rival females as greater competition for resources (Cain et al, 2011). This type of parental investment can protect offspring from conspecifics and deter harassment (Maestriperi, 1998). In many cases, female aggression is

used to defend against infanticide by other females and males (Hrdy, 1979; Agrell et al, 1998). Infanticidal individuals can be motivated by access to resources such as food, mates, and mating sites (Agrell et al, 1998; Hrdy, 1979), and females often deter infanticide through strong aggression (Agrell et al, 1998). Among mammals, female territoriality may be more strongly driven by the prevention of infanticide rather than availability of food (Wolff, 1997; Maestriperi, 1992). This is based on evidence that female mammals are more territorial in the spring and summer breeding season than the winter when food is scarcer (Wolff, 1993). There is also a positive correlation between mammalian taxa that display female infanticide and female territoriality and aggression, and this relationship is seen in carnivores, rodents, and lagomorphs (Wolff, 1997; Agrell et al, 1998).

Hormonal mediation of female aggression

A variety of hormones have been found to mediate female aggression in a fashion similar to males (Rosvall, 2011, Elekonich & Wingfield, 2000). An interesting example comes from *Neolamprologus pulcher*, a cooperatively breeding cichlid fish from Lake Tanganyika in Africa which demonstrates territorial defense in both males and females that is modulated by androgens in both sexes (Walter & Trillmich, 1993). Male cichlids defend a group of shells used as shelter and thus gain access to a harem of females with which to mate. Females show more aggression to female intruders than male intruders because of perceived competition for territory and mate resources (Schradin & Lamprecht, 2000). This aggression was found to be modulated by 11-ketotestosterone in both sexes during intruder simulation, but only females experience elevated levels of testosterone (Desjardins et al, 2006). Another example comes from tropical spotted antbirds, which establish pair bonds and both male and female aggressively defend a territory

both inside and outside of the breeding season (Hau et al, 2004). Among females this aggression is a result of elevated levels of dehydroepiandrosterone (DHEA), a steroid hormone associated with aggression. In this system, it was also found that females are more aggressive towards intruding females than male intruders and that females use status signals to demonstrate hormone levels and fighting ability. In another example, female American goldfinches are highly aggressive with one another and within-sex conflict is mediated by bill coloration which functions as a signal of status (Murphy et al, 2009). This status signal is related to circulating testosterone (Pham et al, 2014).

While female aggression has been studied in a variety of taxa, there are many aspects which remain to be studied. Although there are obvious selective pressures driving female aggression and territoriality, it may be important to examine potential carryover effects from aggression in males (Rosvall, 2011; Lande 1980). In species where male aggression may be selected for, females may become more aggressive because they share the same genetic pool (Ryan & Vandenberg, 2002). Depending on the strength of the carryover effect, it may be important to consider confounding variables when making conclusions regarding selective pressures driving female aggression. It is also important to take into account potential costs of female aggression. Female guppies exposed to competition grew more slowly while in competitive situations than before or after exposure (Borg et al, 2012). In order to compensate for this cost of competition, females increased growth rates after exposure. There are costs to aggression in males that have been well-studied, such as opportunity cost of territorial defense versus foraging, but these studies have yet to be extended to females (Cooper, 1999).

There are many selective pressures that drive the evolution of and maintenance of aggression and territoriality in females. Hormonal and mechanistic controls for aggression in

females are still very understudied, as is female competition for mates. Additionally, female aggression and impacts on offspring survivorship should be studied in multiple contexts.

Preliminary research in the field has shown that there are negative impacts of female aggression on offspring growth and survivorship; female tree swallows with artificially elevated testosterone show more aggression and a resulting lower incubation temperature at the nest, negatively impacting rates of hatching (Rosvall, 2013). Another study performed on rats found that maternal aggression in response to chronic intrusions at the nest limits maternal care and subsequently the growth rate of the pups (Nephew & Bridges, 2011). Further study can elucidate the connections between female and male aggression, the strength of selection on female aggression, hormonal regulation, and potential costs.

Chapter Two: Resource value as a mediating factor of aggression during within-sex competition between female Siamese fighting fish (*Betta splendens*)

Abstract

In order to acquire and defend resources, individuals make decisions about the benefits of acquiring a resource weighed against the potential cost of injury and lost time and energy. Individual investment into aggression is expected to be proportional to the value of the contested resource, and that investment in defense of different categories of resources (e.g. food, mates) will vary depending on the value of each category of resource. Female Siamese fighting fish (*Betta splendens*) competed in dyadic trials with other female bettas in two experiments: one for food of either high or low value (small or large amount of food), and second, for males of either high or low value (large or small bodied male). Aggression was quantified as the number of behavioral displays and physical attacks performed during each competitive interaction. Within a dyad, female bettas expressed more physical aggression when competing over a small amount of food compared to a large amount of food, yet had fewer displays when competing over a small amount of food. Shorter display time and more attacks are consistent with the hypothesis that females perceive a small amount of food as more valuable because the resource may be depleted more quickly. In male stimulus trials, females increased aggression when they were presented with a small-bodied male compared to a large-bodied male. This is consistent with the hypothesis that females exhibit a choice for smaller males, which may pose less threat to females, as they have reduced aggression during courtship compared to larger males. To examine the type of selective pressures driving female aggression, I analyzed the difference between female aggression over food or males. Females showed more aggression when competing over food compared to competing over males, indicating stronger social selection for female

aggression for obtaining food, while sexual selection for acquisition to mates has favored lower levels of aggression in mate-based competition. These results indicate that females modulate aggression depending on resource value, irrespective of the category of contested resource, and that a limitation of food resources appears to be a strong driving factor in female aggression, while competition for access to mates, although present, is less robust.

Introduction

Individuals must weigh the benefits of aggressively acquiring a resource against time and energy cost, as well as against the risk of injury. A resource of high intrinsic value or one that is limited may cause an individual to incur greater risks (Arnott & Elwood, 2008; Rosvall, 2011), and individuals across taxa have been found to invest more into aggression when resource value is high (Enquist & Leimar, 1987), including among hermit crabs (Gherardi, 2006), wasps (Tibbetts, 2008), squid (DiMarco & Hanlon, 1997), red-spotted newts (Verrell, 1986), and sunfish (Dugatkin & Ohlsen, 1990). Variation between individuals in their investment in resource defense indicates that aggressive behavior is not simply guided by intrinsic differences in fighting ability, but that motivation to fight can be an important determinant of aggressive behavior (Arnott & Elwood, 2008; Verrell, 1986). Examination of how the value and limitation of a resource affects an individual's investment in aggression can elucidate the selective factors maintaining aggression and resource defense, and can indicate whether sexual selection is a major selective force maintaining such behavior (when competition is for mates), or whether social selection is a selective force maintaining aggression (Stamps, 1977; Symons, 1968).

While male aggression and defense of resources is well studied, female aggression has not received much attention (Rosvall, 2011). Females gain various fitness benefits through aggression, including during competition to protect offspring (Rosvall, 2011; Agrell et al, 1998; Wolff, 1997), gaining access to paternal care (Breihagen & Slagsvold, 1988; Rosvall, 2011; Yasukawa & Searcy, 1982), competition for territories (Murphy et al. 2009), and competition for food resources (Michels, 1998). One similarity between aggressive behaviors between the sexes is observed during competition for food, where both sexes gain similar payoff from acquiring food (Marra & Holmes, 2001); however, in some cases female need for food is greater than males during offspring production, so females can be more aggressive than males during these periods (Rosvall, 2011). While female aggression is subject to many of the same selective pressures as male aggression, there are also sexual differences in the benefits associated with aggression (Cunningham & Birkhead, 1998). For example, in many taxa, there is unequal parental investment between the sexes wherein only one parent performs the majority of parental care, and in cases where only males invest in parental care (Agrell et al, 1998; Rosvall, 2011), females compete to secure a high quality male or a male that can invest quality paternal care.

Both females and males invest in aggression during competition for the acquisition of food and territories. Limited or high quality food has been found to stimulate competition and aggression among many taxa, including birds, lizards, primates, and fish (Kodric-Brown & Brown, 1978; Stamps, 1977; Symons, 1968, Michels, 1998), and more aggressive individuals are able to acquire and maintain access to a greater proportion of resources (Kodric-Brown & Brown, 1978; Michels, 1998). Large group size may limit available food for an individual and can cause increased competition for food and higher individual aggression (Magurran & Seghers, 1991). For example, female lizards engage in more competition over food when territory

boundaries overlap due to uneven distribution of resources, modulate their behavior based on perceived risk by the competitor (Stamps, 1977). Captive common marmosets were shown to acquire more food resources than males when put into competition because of their higher aggression and apparent increased motivation to search for food (Michels, 1998).

Both females and males are also known to invest in aggression during competition for the acquisition of mates (Sandell, 1998; Walter & Trillmich, 1994; Alcock, 1994; Poole, 1989). Males are well known for their aggression towards same-sex competitors in the mating arena while striving to gain access to multiple females (Andersson, 1994). Females too are competitive in the mating arena, but often competition among females is over mate quality, and not the quantity of mates. Because the quality of a mate can be a limited resource, females often invest in aggression towards female competitors in order to gain access to the best mate, or to monopolize paternal care and investment by a mate (Yasukawa & Searcy, 1982; Desjardins et al, 2006; Sandell, 1998). In dark-eyed juncos and spotted antbirds, females showed more aggression towards female intruders, which compete for paternal care (Cain et al, 2011; Hau et al, 2004). Such female aggression is selected for because male quality and parental care can greatly impact offspring quality and survivorship (Wolf et al, 1988; Slagsvold & Lifjeld, 1994).

In this study, I test whether female betta fish (*Betta splendens*), hereafter bettas, modulate their aggression based on value of a contested resource. To test this, I presented a dyad of females with limited food that varied in quantity between two treatments, and in a separate experiment, presented a dyad of females with a potential mate that varied in quality (male size) between two treatments. I monitored competition within a dyad and predicted that females would invest more into aggression when food resources were limited, and thus easily depleted, compared to when abundant. I also predicted that competition within a dyad would be greater

when they were competing over access to a large male compared to a small male. Female bettas have been shown to exhibit preference for large males (Clotfelter et al, 2006), and larger males were assumed to represent greater mate value because females of many fish species show a preference for large body size in males (Rosenthal & Evans, 1998), which often indicates mate quality (Reynolds & Gross, 1992). By quantifying female aggression in response to high or low value food resource or high or low value male resource, I tested how a female's investment toward a particular resource varied depending on the value of the resource. I also tested what selective pressures may be maintaining female aggression by comparing the level of aggression between dyads when they competed for a non-mate based food resource (implying social selection) compared to when they competed over a mate-based resource (implying social selection (West-Eberhard, 1979; Tobias et al, 2012)). I predicted that females would exhibit more aggression during competition for food than for males because females face competition daily for limited food resources.

Materials and Methods

Study species

Betta splendens are endemic to Southeast Asia where they have traditionally been used in staged fights and have been subject to artificial selection for hyper-aggressive tendencies, coloration, and long fins for centuries (Jaroensutasinee & Jaroensutasinee, 2001a; Monsives et. al. 2009). Bettas are highly aggressive, territorial, externally fertilizing fish that have a long history as a study species for aggressive behavior and communication signaling (Doutrelant & McGregor, 2000; Jaroensutasinee & Jaroensutasinee, 2001a; Meliska & Meliska, 1976; Meliska et al., 1975). Bettas aggressively display for extended periods, and aggression often escalates

into aggressive biting and tail slapping (Jaroensutasinee & Jaroensutasinee, 2001a; Goldstein, 1975). Both male and female bettas engage in similar aggressive displays. These includes fin flares where all fins are unfurled and held erect away from the body in order to appear larger, gill flares where the operculum is pushed forward away from the body making the betta's head appear larger, chasing, circling, "tail slapping," strikes, and biting (Braddock & Braddock, 1975, Todd et al, 2008). Although male betta aggression has been well studied (Clotfelter et al, 2007; Meliska & Meliska, 1976; Jaroensutasinee & Jaroensutasinee, 2003), less is known of female aggression in this species. Female bettas are known to be aggressive towards other females, particularly when first establishing a hierarchy that allows predictable access to resources (Elwood & Rainey, 1983; Goldstein, 1975; Braddock & Braddock, 1955), and individual levels of aggression are often stable (Todd et al, 2008).

In the wild, among the ancestral wild-type bettas, males are highly territorial and create and defend a bubble nest where females deposit eggs after courtship (Jaroensutasinee & Jaroensutasinee, 2001a; Monsives et al, 2009). Males then aerate and protect the eggs until they hatch after approximately 36 hours, care for and protect the fry for 5-7 days after hatching, and though they care for one female's brood at a time, may have 2-3 broods during a breeding season (Jaroensutasinee & Jaroensutasinee, 2003).

General Approach

I acquired bettas from a local breeder for use in the study. All fish were housed in individual 1.5 liter tanks connected through a shared water filtration system. Visual dividers between tanks prevented fish from seeing their neighbors.

Before I created competitive dyads of females, I photographed all fish and sorted them into color (blue, green, red, black, brown, etc.) and size groups (see below). I collected color and size measurements by laying individuals out on a moistened paper towel in a petri dish and using a ruler laid against the fish to visually take standard length measurements. At this time I also measured mass. Care was taken not cause undue stress to the animals; bettas were out of the water for a maximum of 30 seconds. Females were sorted into three different size groups by taking both length and mass into account small <43mm and less than 2.0g; medium 43 - 55mm and less than 2.0g, large >55mm or more than 2.0g). For the mate competition trials, males were measured using the same protocol and were sorted between color (red, green, orange, blue, etc.) and size (small $\leq 1.4\text{g}$; large $>1.4\text{g}$). This was done for the mate competition trials so that females would be exposed to males of similar color but opposite size category during the two trials. Male aggression and female preference for males have been associated with certain color morphs in bettas (Clotfelter et al, 2007), so it was important to match males for color category.

Fish were held in individual tanks for 14 days before trials began. During this period, females were fed four floating food pellets (Omega One Natural Protein Formula, Betta Buffet) once a day placed in the center of “training ring” which consisted of a foam ring which kept floating food pellets within a circle approximately half an inch in diameter. This trained the fish to seek food placed in the center of the ring, and the ring was of such a small size that it could later be monopolized by an individual female (personal observation).

One hundred twelve females were used for the experiments; females were randomly assigned one of two stimulus categories, either food or male stimulus. Females were used for two competitive interactions while competing for one type of resource (food or mate), and each stimulus (i.e., small or large amount of food) was presented in a separate trial. Females competed

with the same competitor only once (e.g., females A versus B competed for a small amount of food, while females A versus C, and B versus D competed for a large amount of food) to avoid any experience effects in the second trial. Females that competed in the food-competition trials did not compete in the mate-competition trials.

Female-female competition over food resources of different value

Twenty-eight dyadic competitions were staged with a small food stimulus and twenty-eight trials were staged out with a large food stimulus. For the small food stimulus trial, five floating food pellets were placed in a training ring and the fish were allowed to compete for access to the food (see below). For trials with large food stimulus, ten floating pellets were placed within a training ring. Trials were balanced so that half of the trial dyads competed for small amounts of food before large amounts of food.

Forty-eight hours before the competitive trials, females were deprived of food to motivate and standardize hunger. Before the dyad was allowed to compete, I placed both fish in visually isolated sections of a 10 gallon tank for a 10-minute acclimation where the fish could not see one another or see the food ring (fig. 1). After the ten-minute acclimation period, I removed the dividers between the females and the food stimulus. The females were then able to interact with each other and the food stimulus freely. Trials were videotaped for twenty-minutes. After a female experienced a competitive trial, it was separated and placed back into its individual tank to rest for two days. After two days, these females were subject to the same food deprivation as specified above and were again allowed to compete, but were subject to the opposite treatment (small amount of food, versus large amount of food).

Female-female competition over potential mates of different value

Twenty-eight dyadic trials were conducted where females were allowed to compete for a small-bodied male stimulus fish and another twenty-eight trials were conducted where females competed for a large-bodied male stimulus fish. Males of the same color morph, yet of different size-classes were assigned to the two trials. In the two male-competition trials, females were allowed to compete for a small and large bodied male against a novel competitor. Half of the females were exposed to the small-bodied male first, and half were exposed to a large-bodied male first to reduce any potential training effect. When each trial was over, the females were returned to their individual tanks, and three days later the male-competition trial was repeated with a male of the opposite body size and females competed against a different female.

The trial tank was set up with a divider of tinted window film placed roughly a third of the length down the tank. There was a light placed above the male section of the tank, thus making the tinted window film function as a one-way screen. This allowed the competing females to perceive the presence of a male, but the male could not see or interact with the females. Before the trials begun, a pair of foam dividers was placed in a T-shape on the larger end of the tank that prevented the females from seeing one another or from seeing the male (Fig. 2).

To begin the behavioral trial, females were acclimated to the tank for a period of ten minutes and then the foam dividers were removed. The female bettas were thus able to interact freely with each other for the twenty-minute trial period. As above, the trials were videotape for later behavioral analysis.

Behavioral Analysis

Video footage was analyzed to quantify behavioral displays and attacks. Displays were operationally defined as fin flare, gill flare, chase, and circling behavior, and attacks were defined as tail slapping and biting. I began all video analysis at the moment in which the dividers were removed between the females. I did not record behavior for a one minute period after the initial removal of dividers in the tank to allow the females to register the presence of each other and the stimulus. I then recorded data for the following fifteen minutes of video in which the females were competing. Scoring of aggressive interactions were conducted by quantifying the number of displays and attacks performed by each fish over a ten-seconds observation period, followed by twenty-seconds pause, and then repeating this cycle for the duration of the 15 minute recording period. Videos were analyzed twice so that each competing female could be scored individually.

Data analysis

All analyzes were conducted in JMP (version 12). I assessed individual change in aggression between stimuli of different value. I used a two-way repeated measure ANOVA with stimulus type as a factor in the model. Food-competition and mate-competition trials were analyzed separately.

I also quantified overall aggression summed for each dyad so I could compare overall aggression when females faced different resources of different values in each experiment (small versus large amounts of food; large versus small bodied male), I analyzed all behavior observed in the tank, irrespective of which female was participating. Independent t-test were used to

analyze differences in overall aggression between a small amount and large amount of food, and a separate test analyzed differences in overall aggression between small and large males.

In addition to the above approaches, I analyzed difference in the aggressive behaviors of the “winner” and "loser" of each dyadic competition. I defined winners as the female with the higher summed aggression score of the dyad and the loser as the females with the lower summed aggression score. This analysis allowed me to assess whether changes in overall tank aggression (the above analysis) were driven by an increase or decrease in aggression of the winner or loser. This was done because overall tank aggression could have been influenced by different reactions of the dominant (winner) or subordinate (loser) females. For, example, an apparent increase in aggression could have occurred if winners and losers both invested more aggression, or it could have occurred if the winner invested far more aggression than the loser. Independent t-test was used to assess differences in mean aggressive scores of winners and losers.

To asses differences in overall behavior when food was the contested resource versus when males were the contested resource, I summed overall tank aggression performed towards each stimulus type and compared the summed scores using independent t-test (pooled aggression scores during competition for small amounts and large amounts of food scores compared to pooled scores from small and large-bodied male trials).

Some trials had to be discarded because of fish death between trials, or errors in tank setup where the male was aware of, and signaling to, the females. Four trials from the food stimulus experiment and six trials from the male stimulus trials had to be discarded for the above reasons, yielding twenty-four trials of the food stimulus and twenty-two trials of the male stimulus.

Results

Competition for food trial

When looking at differences in how individuals behaved when they encountered different stimuli, females invested significantly more in attacks when there was a small amount of food over a large amount of food ($F= 7.9397$, $df= 1$, $P=0.0072$, $n=46$; fig. 3). Individual fish did not change their displays significantly between both small and large food ($F= 1.030$, $df=1$, $P=0.316$, $n=46$; fig. 3).

When comparing overall aggression in a tank (scores of both females pooled), females invested more in attacks when presented with a small food stimulus than a large food stimulus ($t= 2.035$, $df=33.460$, $p=0.05$, $n=24$). Females engaged in significantly more display behavior when presented with a large food stimulus than when presented a small food stimulus ($t=-2.575$, $df=46$, $p=0.013$, $n=24$).

When looking for differences between winners and loser behavior, there was no significant difference between winners and losers between small and large food stimuli in either attacks ($t=0.178$, $df=46$, $p=0.859$, $n=24$) or displays ($t=-0.686$, $df=46$, $p=0.496$, $n=24$).

Competition for male

Females attacked more when there was a small male rather than a large male ($F=9.0868$, $df=1$, $P=0.0046$, $n=38$; fig. 4). In contrast, there was no difference in female display behavior when the dyads competed for small- versus large- bodied males ($F=0.5781$, $df= 1$, $P=0.452$, $n=38$; fig. 4).

When comparing overall aggression in a tank, females invested more heavily in attacks when a small male was present over a large male ($t=2.497$, $df=21.251$, $p=0.021$, $n=22$). There

was no significant difference in the amount of displays performed between the females that were presented with either a small- and large-bodied male stimulus ($t=0.542$, $df=42$, $p=0.591$, $n=22$).

Winners demonstrated significantly more attacks than losers in small-bodied male stimulus trials when compared to large-bodied male stimulus trials ($t=2.233$, $df=30.172$, $p=0.033$, $n=22$). There was no difference in displays between winners and losers in either male stimulus ($t=-0.343$, $df=42$, $p=0.733$, $n=22$).

Behavior differences between food and male stimuli

When comparing aggression among dyads when they competed for food or mates (using pooled data from both low- and high-value resources), females showed both more attacks ($t=2.328$, $df=30.377$, $p=0.027$; fig. 5) and displays ($t=2.115$, $df=44$, $p=0.040$; fig. 5) in food stimulus trials when compared to male stimulus trials.

Discussion

Because motivation can strongly influence the amount an individual invests into resource defense (Arnott & Elwood, 2008), I hypothesized that female bettas would modulate their aggression depending on the value of a contested food or mate resource. I predicted that female bettas would engage in more aggression when food resources were perceived to be limited and that females would invest in more aggression and mate defense when a large-bodied male was present rather than a small-bodied male. I found that female bettas modulate their attacks and display behavior when presented with different amounts of food: when a small amount of food is present, females engaged in significantly more attacks, thus supporting the hypothesis that females perceive a limited resource as more valuable. Interestingly, even though attacks

increased, there was less display behavior in the presence of a small amount of food. Because of an opportunity cost related to display behavior, females may be investing less into display and resorting to an escalated fight when faced with a quickly diminishing resource. In other words, limited food may motivate females to strategically increase defense to a high level of aggression, and thus forgo less risky display behavior. In contrast, females may have invested more into display (and so spent less time being aggressive) in the presence of a large amount of food to reduce the risk of injury, while at the same time maintaining some access to the food. These results support the hypothesis that females engage in more aggression when resources are limited.

These same patterns were found when comparing overall tank aggression during competition for food: more attacks were performed when presented with small amounts of food, and more displays were performed when presented with large amounts of food. When I compared the difference in winner – loser scores between small and large food, there was not a significant difference in either display behavior or attacks. Therefore, both females are either heightening or lowering their display and attack behavior. In these cases, it appears that both females perceive food as a similarly valuable resource and are moderating their behavior based on whether it is limited or not.

In male competition trials, results were similar but in the opposite direction: females invested more in attacks when a small male is present. Also, I found that females do not modulate their display behavior between different sized male stimuli fish. These results are in the opposite direction than that predicted by our hypothesis, which was based on the assumption that the larger male would be perceived as higher quality. Instead, these results may be explained if females prefer small-bodied males because small males pose less risk of female injury during

mating. Previous research has indicated that mating in this species can be highly aggressive and can cause female injury (Todd et al., 2008; Dzieweczynski et al, 2014). Additionally, Dzieweczynski (2014) found that female bettas showed a preference for males based on observed interactions between males, preferring the less aggressive male. Thus it seems possible that the smaller male represented the preferred male, and if true, this would be consistent with our results that females increase aggression towards the resource of greater value.

These same patterns were found when comparing overall tank aggression during competition for mates: more attacks were performed when presented with small males, and there was no difference in the amount of displays performed when presented with small or large males. When I compared the difference in winner – loser scores, winners invested significantly more in attacks when there was a small male present rather than a large male. This indicates that the higher overall tank aggression score was driven by changes in the dominant versus subordinate's behavior, wherein the winner increases her investment in aggression while the loser lowers her aggression. It remains unclear, however, why there would be such a bias towards winners investing more into aggression in this mate-based competition, yet not during the food-based competition. It is possible that during competition for mates that female persistence in competition is a strong determinant of who gains access to the male, but this hypothesis must be explored further.

A comparison of the display and attack scores between food and male stimuli indicates a larger investment in displays and attacks during competition for food compared to a mate. This may indicate that female territorial and aggressive behavior is highly beneficial because it can provide access to food resources and regular access to food is a strong determinant of fitness (Braddock & Braddock, 1955). Despite the fact that bettas can survive for long periods of time

without food (Haller, 1991), female bettas face daily competition for food. In contrast, the reduced investment in competition for males may be explained because, in comparison to food resources which can be quickly depleted, males are very territorial and may remain in one location in the wild with several males around them, thus reducing the limited nature of gaining access to a mate resource in this species (Jaroensutasinee & Jaroensutasinee, 2001a). It is interesting to speculate that it would be favorable then for a female in poor body condition to try and avoid a fight over a male, while a healthy female can afford to choose a male based on factors other than availability.

Greater emphasis should be made on studying the factors that motivate female aggression because each sex is faced with different competitive arenas and thus faces different selective pressures. My results indicate that female bettas have evolved an intricate method for varying aggression based on perceived value of resources, and that they respond to both subtle differences in resource quality, as well as categories of resources that they are competing over. As a whole, these results indicate that female motivation to fight play an important role in their investment into aggression, rather than simple innate individual differences in aggression. Further study can help elucidate how individuals value a resource and can shed light on the selective pressures driving the evolution and maintenance of female aggression.

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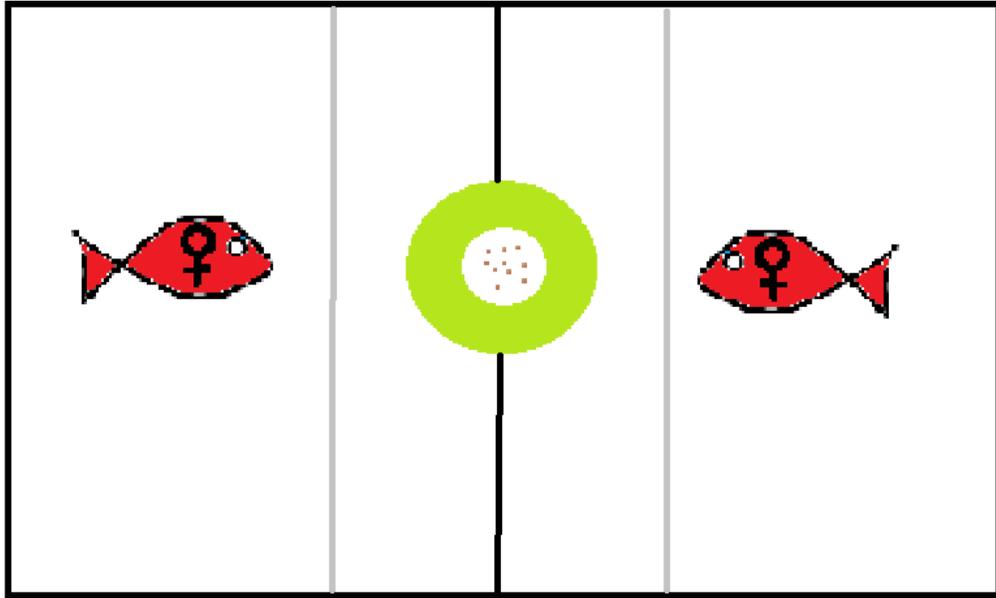


Figure 1: Competition for food stimulus trial setup

In the center of the glass, ten-gallon tank is a foam food “training ring” suspended by thread attached to the sides of the tank. The training ring consolidates the food in a small, monopolizable area. Gray lines indicate foam dividers that were in place for a ten-minute acclimation period before the trial began.

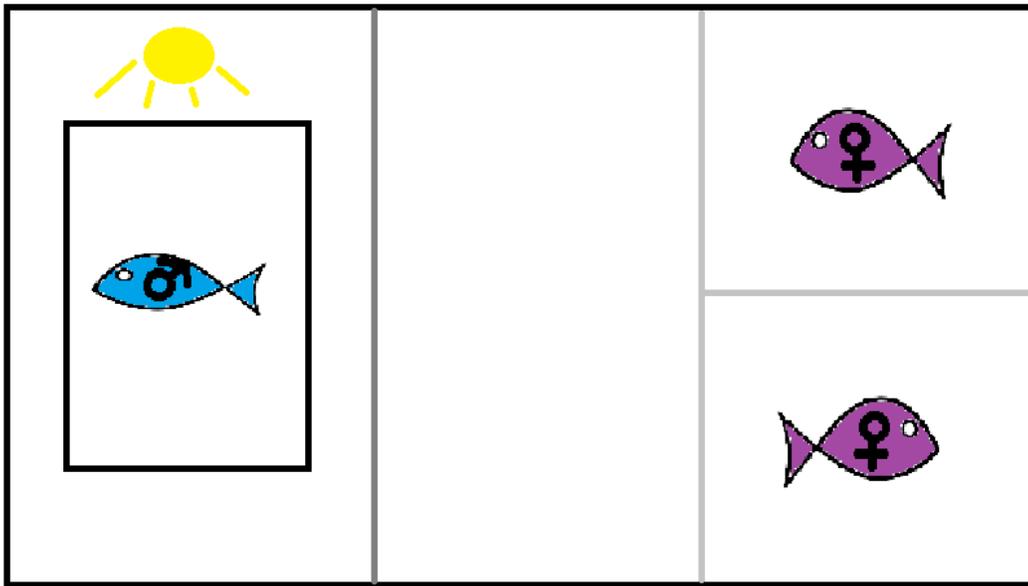


Figure 2: Competition for male stimulus trial setup

The glass ten-gallon tank is divided the male section from the female section by a piece of tinted window film. A male stimulus fish was placed on one side of this divide with a floodlight suspended above the tank that prevented the male from seeing the females. Gray lines indicate foam dividers that were in place for a ten-minute acclimation period before the trial began.

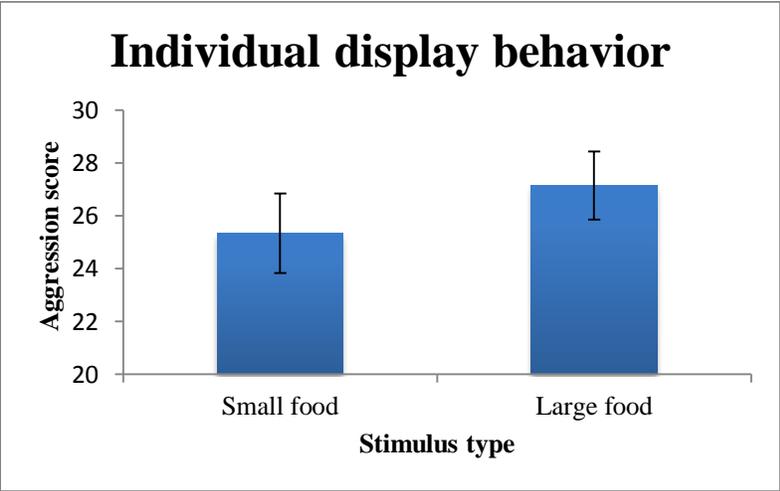
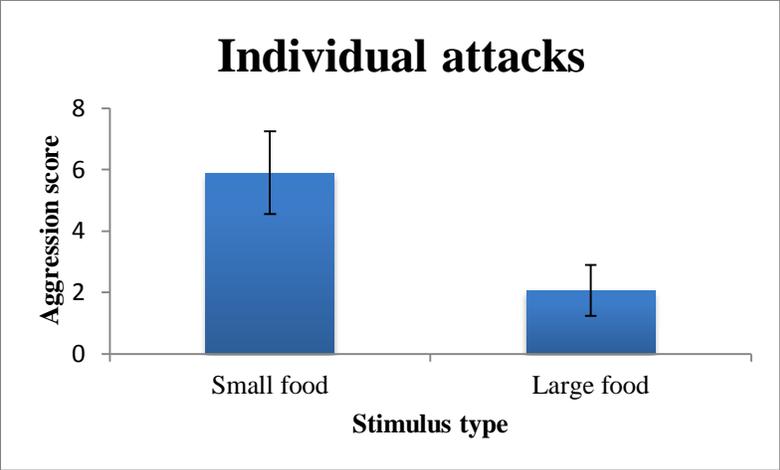


Figure 3
Average attack scores of individual female bettas exposed to a small amount of food versus a large amount of food (top), and the average display behavior of females exposed to a small amount of food and a large amount of food (bottom).

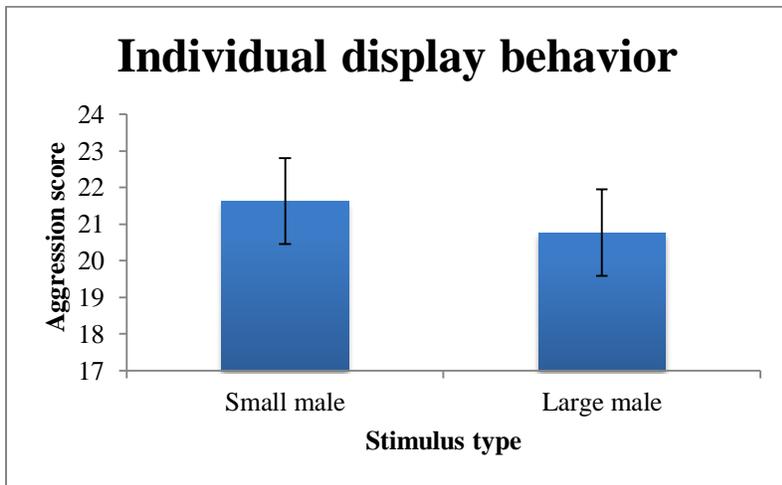
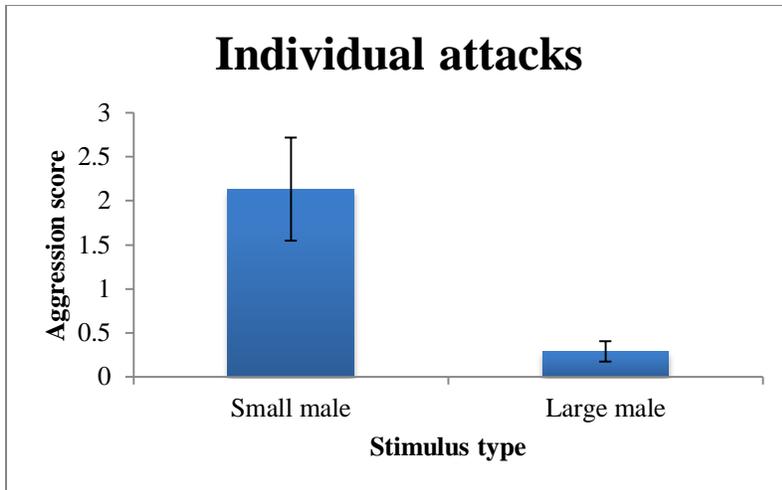


Figure 4

Average attack scores of individual female bettas exposed to a small-bodied male versus a large-bodied male (top), and the average display behavior of females exposed to a small-bodied male and a large-bodied male (bottom).

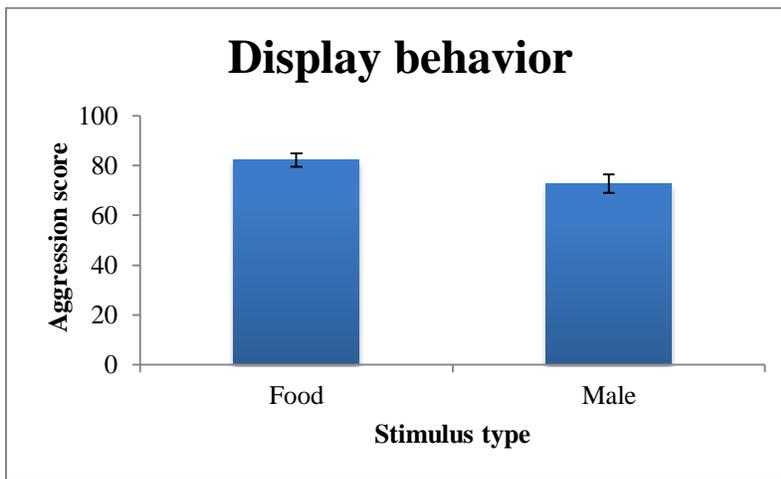
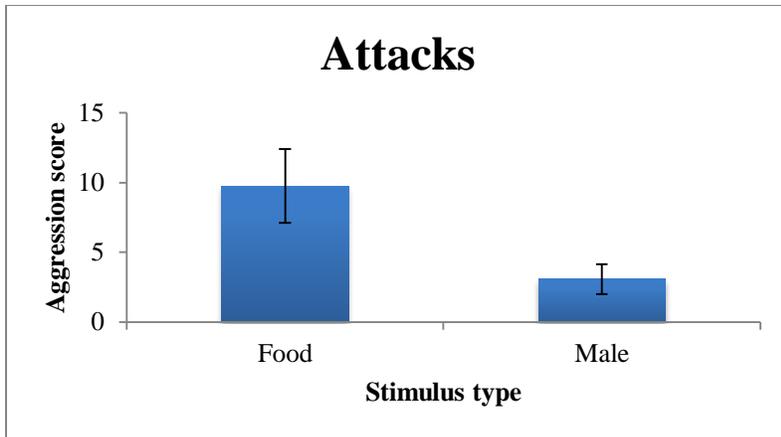


Figure 5

Average summed attack scores of dyadic aggression of female bettas exposed to a food stimulus (small amount of food and a large amount of food) and a male stimulus (small-bodied male and large-bodied male) (top), and the average summed display behavior of females exposed to a to a food stimulus (small amount of food and a large amount of food) and a male stimulus (small-bodied male and large-bodied male) (bottom).