Risk-sensitivity in male Siamese fighting fish Betta splendens

Baine B. Craft

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RISK-SENSITIVITY IN MALE SIAMESE FIGHTING FISH,

BETTA SPLENDENS

MISSOULA, MONTANA, USA

by

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Risk-sensitivity in male Siamese fighting fish, *Betta splendens*

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The purpose of the current experiments was twofold. In the first experiment, the purpose was to provide an empirical comparison of three discrimination methodologies proposed for use in choice preparations. Subjects were thirty-five, healthy, adult male Siamese Fighting Fish (*Betta splendens*). Subjects were randomly assigned to one of three discrimination groups: a Direction Group (using left or right as discriminative stimuli), a Color Group (using red or green as discriminative stimuli) and Bubble Group (using the presence or absence of air bubbles as discriminative stimuli). For all three Discrimination groups, subjects chose between one or three food pellets in a submerged T-maze. The results from Experiment 1 revealed a statistically significant preference for the three pellets of food over one pellet of food only for the Bubble Group. Of particular note is the effect size and observed power obtained for the Bubble Group. The Bubble Group is the only group which supports a reasonably powerful test for discrimination, given a sample size of 12 subjects and a 0.94 effect size.

Using the results from the Experiment 1 to aid in discrimination, the purpose of the second experiment was designed to gain an understanding of choices made between variable and constant food rewards (i.e. risk-sensitivity) in *Betta splendens*. To explore risk-sensitivity using a food stimulus, instrumental conditioning of choice behavior was used to determine choice bias in male *Betta splendens* (*n* = 15) between a variable food option and a constant food option. The results from Experiment 2 revealed no significant difference in choice bias. A discussion of sensitivity to food amount in *Betta splendens* and suggestions for future research is provided.
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General Introduction

Research directed toward understanding the relationship of choices made between variable and constant rewards has been labeled risk-sensitivity (Smallwood, 1996). Alterations in foraging strategies, as the result of variability or delay to reward, have been examined across a variety of organisms (see Kacelnik & Bateson, 1996 for a review). Although many studies have been conducted, a comparative model or theory that accurately describes risk-sensitivity remains elusive. However, several models or theories have been proposed to explain the relationship between foragers’ shifts in choice due to extraneous variables.

Optimal Foraging Theory (OFT), specifically molar maximization models, describe a foraging strategy in which an organism seeks to choose a reward or food source that provides the maximum amount of food per unit time (Pyke, Pulliam, & Charnov, 1977). When presented with an option to choose between two rewards (a constant reward and a variable reward) yielding the same amount, OFT suggests that a forager will be indifferent or choose the constant reward option. OFT explains such choices as the result of a forager seeking to minimize delay to reward and maximize reward amount, both of which can be experimentally manipulated. However, in this manuscript, focus will be placed on reward amount and delay such that delay to reward will be discussed as a forager choosing a variable reward option that returns a small reward amount over several trials or, in other words, a run of bad luck. In this situation, the delay would increase until the variable reward option returned a large reward amount.

While OFT purports to account for foraging decision in animals, several problems with the OFT model become apparent when a forager makes choices that involve variable
rewards. First, OFT does not take into account situations that prevent an organism from performing optimally (Stephens & Charnov, 1982). Thus, failures in optimal performance may be the result of restrictions in the environment or the organisms’ evolutionary background (Pyke, Pulliam, & Charnov, 1977). Second, OFT states that a forager seeks to minimize delay in reward. If OFT is an appropriate comparative model to describe foraging behavior, foragers receiving a mean reward amount should be indifferent or choose a constant reward. However, the OFT has been called to question as an adequate description of foraging behavior by experiments in which subjects displayed a choice for a variable reward (e.g., Caraco, Martindale, & Whitman, 1980; Caraco, 1981; Caraco, 1982).

In experiments by Caraco et al., (1980), Caraco (1981), and Caraco (1982), subjects responded differently to variations in food sources as a result of the subject’s fitness. For example, Caraco et al., manipulated the food intake of yellow-eyed juncos (Junco phaeonotus). Subjects denied the number of seeds per minute required to maintain fitness, chose a variable food option as opposed to a constant food option. Subjects provided with the number of seeds per minute required to maintain fitness, chose a constant food option as opposed to a variable food option. In other words, subjects with low fitness or a low energy budget chose the variable food option and subjects with a stable fitness or stable energy budget chose the constant food option. The choice of a variable option under any condition or level of fitness deviates from the predictions asserted by the OFT. Thus, the development of a theory was needed to provide a more adequate description of foraging behavior in situations where a variable option is preferred.
Risk-sensitive Foraging Theory was developed to explain a choice bias directed toward a variable or constant option under certain circumstances (i.e. where OFT fails to explain the choice of a variable option). If an organism displays a bias in choice, the organism is said to be risk-sensitive (Smallwood, 1996). For example, as described in Caraco et al., (1980), a new problem arose when subjects’ fitness and energy were manipulated. Due to the manipulation of subjects’ fitness and energy, subjects’ choice shifted between a constant reward and a variable reward. Therefore, choice behavior could not be predicted accurately using OFT. Using Jensen’s inequality, researchers (e.g., Houston, 1991; Stevens, 1981; Smallwood, 1996) could predict the shift between constant and variable rewards by describing the relationship between fitness and energy as being a non-linear function.

In Jensen’s inequality, the function $y=f(x)$, where $y$ represents fitness and $x$ represents amount of food obtained, demonstrates the interaction between fitness and obtained food. In other words, as income or the amount of food obtained ($x$) increases or decreases, fitness ($y$) increases or decreases respectively. The prediction of a forager’s expected fitness can be denoted as $E(y)=f[E(x)]$ such that $f[E(x)]=E[f(x)]$ where the function of $x$ is linear and can be explained by Optimal Foraging Theory. However, Jensen’s inequality states that the function of $x$ is non-linear or $f[E(x)]$ cannot equal $E[f(x)]$ (see Figure 1a and 1b; Smallwood, 1996).
Figure 1a and 1b. Jensen’s inequality.
Figure 1a and 1b illustrate fitness on the x-axis and income on the y-axis. In Figure 1a, A (constant option) yields a higher degree of fitness than B (variable option). However, in Figure 1b B yields higher fitness.

For example, given a hypothetical foraging bout with one choice option delivering a constant income and another delivering variable income, Jensen's inequality predicts that a bias will occur due to potential changes in the forager's fitness. If fitness accelerates negatively with respect to income, Jensen's inequality predicts that the forager will choose a constant return. However, if fitness accelerates positively with respect to income, Jensen's inequality predicts that the forager will choose a variable return. While Optimal Foraging Theory fails to account for such a non-linear relationship, the Daily Energy Budget Rule (DEB) rule assumes that the relationship between fitness and energy is non-linear.

The DEB rule makes the assumption that foraging bouts provide an organism with an amount of energy to survive until the organism can forage again, given an interruption in foraging (for example, overnight) (Houston, 1991). If the foraging bout begins with an organism possessing some initial energy reserve \( x \) then the organism will have to forage for a specific amount of time \( T \) at some mean rate \( \mu \) until the amount of energy required \( R \) to survive is obtained. Thus, a forager with a positive energy budget is expressed as \( x + \mu T > R \) and a forager with a negative energy budget is expressed as \( x + \mu T < R \).
As a forager reaches a negative energy budget, the DEB rule describes the organism as seeking a reward with the greatest amount of return despite the variability or delay of the reward (risk prone; see Figure 1b). On the contrary, organisms with a positive energy budget will choose a constant reward in order to maintain a positive energy budget state (risk averse; see Figure 1a) (Caraco, 1980). For example, Barnard and Brown (1985) manipulated the food intake of common shrews (*Sorex araneus*). In their experiment, common shrews were kept at a negative energy budget by depriving them of the number of worms required to maintain a stable energy budget. Shrews in a negative energy budget chose a reward with the greatest amount of return despite the variability or delay to the reward, in other words, a risk prone foraging strategy. Contrary to the shrews with a negative energy budget, shrews given the number of worms to maintain a positive energy budget chose a constant reward in order to maintain that economic state, in other words, choosing a risk averse foraging strategy. Due to organisms’ (such as shrews) susceptibility to variations in energy budget, shifts in choice due to economic state, explained by the DEB rule, can be accurately predicted using the z-score model.

The z-score model predicts that the forager will attempt to minimize the probability of a deficiency in calories (Stephens, 1981). In other words, the z-score model describes an organism (usually small avian or mammalian species) in a positive or negative energy budget, as trying to postpone starvation. If the forager is attempting to minimize a deficiency in calories, the forager will be attentive to the mean return and the variability of rewards. If presented mathematically, the reward required for survival ($R$) is subtracted from the mean reward ($\mu$) and is divided by the standard deviation of the
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reward (\(\sigma\)) or \(z = (R - \mu)/\sigma; \mu = R - z\sigma\) (Stephens & Paton, 1986). Despite predictions made by the z-score model and empirical validations of the DEB rule, researchers (e.g., Gibbon, 1977; Kacelnik & Bateson, 1996) have argued that the DEB rule fails to sufficiently describe foraging behavior.

Kacelnik and Bateson (1996) argued four points in which the DEB rule fails to account for various phenomena demonstrated by foragers. First, the DEB rule, as presented by Stephens (1981), assumes that a forager will choose a strategy, such as risk averse, and will not change that strategy for the remainder of the day. The DEB rule fails to take into account the possibility of the forager changing strategies as the organism’s energy budget change, which is a likely possibility if the organism receives a large return as the result of choosing the variable option. Second, the DEB rule assumes that some minimum caloric level must be met before foraging is interrupted by instances such as nightfall. The DEB rule does not take into account the possibility of a forager that is required to continuously forage such as several small mammalian or avian species. For example, if a forager does not achieve a positive mean net gain, the forager is forced to continue foraging at the risk of starvation. The third problem, the DEB rule fails to account for any energy expenditure directed toward any behavior other than foraging. For example, the DEB rule describes a static environment with no competing behaviors such that an organism required to care for young, defend a territory, compete for mates or other behaviors that would deplete energy reserves might not be accurately described. Finally, the DEB rule fails to explain variations in delay to reward. For example, if a forager experiences a run of bad luck by choosing the variable option (returning only a small reward amount), the delay to reward will increase until the variable option returns a
large reward amount. In light of these problems with the DEB rule, Scalar Expectancy Theory (SET) was proposed as another plausible explanation for foraging behavior.

Where the DEB rule explains a forager's choice based on that organism's energy budget, the SET describes the forager as choosing a reward option based on two principles. First, a forager seems to make a choice based on the expectation of a reward where the expectation of a reward can be described as an increase or decrease in anticipation for the reward. This anticipation or expectation is contingent on the delay to reward and could be expressed as an exponential function but, if delay to reward becomes lengthy, the reward value depreciates or is discounted. In other words, expectation can be described as increasing hyperbolically as delay to reinforcement increases (Gibbon, 1977). For example, if delay to reward is relatively low, then the discounting value will be relatively low. However, as delay to reward becomes lengthy the reward value will eventually begin to depreciate; therefore, the discounting value increases. Second, SET explains a forager's choice based on the value assigned to a reward option (Kacelnik & Bateson, 1996). To discuss the value assigned to a reward option, a description of Weber's Law is necessary.

Weber's Law states that any noticeable difference in an event produces a record of the event. The difference between events is referred to as a Just Noticeable Difference. In light of Weber's Law, SET explains an organism choice, given the option between two rewards that deviate a Just Noticeable Difference, as being contingent on the differential value associated with each option. For example, the forager associates a value with a reward based on the dissimilar quality or quantity of the two reward options. If a forager
associates a greater value to one reward option over another, the organism should choose the more valuable reward as predicted by SET.

SET predicts that an organism will associate a higher value with a food option that returns a constant quantity of food with a constant delay over a food option that has a variable quantity and constant delay. On the contrary, an organism will associate a higher value with a food option that returns a variable reward when delay to reward is variable over a food option that has a constant return and a variable delay (Kacelnik & Bateson, 1996). Therefore, a forager's behavior is not completely contingent on nor can it be explained by the organism's fitness or energy budget, but by the forager's record of events (events such as delay to reward or variability in reward) in which a desirable reward was received (See Figure 2). Several experiments provide evidence to support the predictions made by SET (e.g. Bateson & Kacelnik, 1995a, 1995b, 1997; Kacelnik & Bateson, 1996).

Figure 2. Scalar Expectancy. Figure 2 illustrates expectation on the y-axis and delay to reward on the x-axis.

All the aforementioned theories or models provide plausible explanations for the proximate causes of variation in foraging behavior, but researchers have failed to agree on one theory or model to describe risk-sensitive foraging. Proximate causes for risk-sensitive behavior in various species can be determined using these theories or models; yet, these proximate causes give no indication of the ultimate causes for foraging
strategies. By conducting further studies using different species, the potential to generalize current theories to other behaviors or species, in an attempt to determine the ultimate causes or overarching characteristics of organisms exhibiting foraging strategies involving risk-sensitive behavior, becomes more probable. In addition, by making these comparisons and generating proximate explanations, development of a unified theory of foraging becomes more probable. However, as previously mentioned, further studies must first be conducted in many different areas and across many different species.

Comparatively, numerous studies have been conducted with a variety of avian and mammalian species, however, few studies have been conducted to determine risk-sensitivity in aquatic species. In light of the relatively small number of studies involving risk-sensitive foraging in aquatic species, Siamese Fighting Fish (*Betta splendens*) would make an excellent model for several reasons. First, a wealth of information is available on this species based on research investigating responding under a variety of situations. Second, the ability to mirror previous studies that investigated risk-sensitivity in other organisms using food reinforcements would be possible. Third, due to the unique species-specific reproductive behaviors of male *Betta splendens* (Betta), the investigation of reinforcers other than food would be possible. Finally, by using male Betta as subjects to explain the properties governing risk-sensitive behavior, the generality of risk-sensitivity could be expanded to include a greater number of aquatic species, as well as, a reinforcer other than food.

Despite the potential benefits of studying sensitivity to risk in male Betta, no studies have examined the effects of the methodologies used to study choice in Betta, thus creating a problem for examining risk-sensitivity in male Betta. Given the subtle
differences in choice options in risk-sensitivity preparations, an examination of potential methodologies that would enhance discrimination would be informative. Therefore, the purpose of the first experiment was to examine the effects of various discriminative stimuli on choice in order to determine an acceptable methodology with which to study risk-sensitivity in Betta.

Experiment 1: The effect of various discriminative stimuli on choice behavior in male Siamese fighting fish (*Betta splendens*)

Investigation of animal choice behavior, across a variety of choices dimensions, has been based largely on the use of a relatively uniform set of methods and procedures. For example, procedures for studying self-control and impulsivity have been adapted for a variety of species, including rats, pigeons, monkeys, and humans, so that a comparative analysis can be conducted with little concern that differences observed are likely due to procedural variations. In terms of investigating choice behavior, a potentially instructive comparison to the aforementioned species would be that of fish. While an extensive literature on choice behavior in a variety of fish is available, the procedures and methods used to examine choice in fish is varies considerably across species and few are consistent with the uniform methods used with the aforementioned species. Thus, due to the procedural variations, direct species comparisons are difficult to establish.

For example, a variety of studies have focused on the role of spatial memory and the use of landmarks in the foraging behavior of several species of fish (e.g. Hughes & Blight, 1999, 2000; Humphries, Ruxton & Metcalfe, 1999; Milinski & Regelmann, 1984; Odling-Smee & Braithwaite, 2002; Rodriguez, Duran, Vargas, Torres, & Salas, 1994; Roitblat, Tham & Golub, 1982; Warburton, 1990). In these investigations, subjects were
given the opportunity to view a landmark or cue while foraging for food in various environments ranging from home tanks to radial arm mazes. Results from these studies suggest that stimuli, landmark/cues, can be associated with specific food patches.

Similarly, studies have examined the effects of conditioned stimuli on learning in fish. In numerous studies (e.g. Hollis, 1984, 1997; Hollis, Dumas, Singh & Fackelmann, 1995; Hollis, Pharr, Dumas, Britton & Field, 1997), Blue Gaurami (*Trichogaster trichopterus*), given the opportunity to view a red light that immediately followed the presentation of a conspecific, were more aggressive (and, in turn, became dominant) than subjects not given the opportunity to view a red light. In addition, several operant experiments (e.g. Chase, 2001; Wirth, Lattal & Hopko, 2003; Zerbolio, 1984, 1985a, 1985b; Zerbolio & Roaylty, 1983) have addressed the use of conditioned stimuli to control the rate of responding in fish. For example, Wirth, Lattal and Hopko (2003) demonstrated changes in responding in Siamese Fighting fish (*Betta splendens*) in the presence or absence of air bubbles that indicated the availability of an operant response.

Finally, a limited number of discriminative stimuli have been used in instrumental conditioning preparations to cue the presence of a reinforcer in fish (Bronstein, 1986; Hogan, 1974; Zerbolio & Wickstra, 1979, 1980). For example, Bronstein (1986) used the colors red and green to cue the presence of another male Betta. However, Bronstein noted a choice bias directed toward the red discriminative stimulus. Similarly, Hogan (1974) used the colors black and white to cue the presence of a mirror image of male Betta and food. In addition, Hogan also noted a choice bias directed toward one color, the black discriminative stimulus.
Given the aforementioned experiments and the numerous instrumental conditioning studies that have examined choice in male Siamese Fighting Fish, *Betta splendens* (Betta), male Betta would seem an obvious choice with which to study the effects of various discriminative stimuli on choice. An additional benefit to the study of discriminative stimuli in Betta would be to provide a foundation from which to study choice bias in relation to food. For example, no research involving Betta has addressed questions concerning choice, where choice options were different quantities of food. Food manipulation may be particularly powerful in Betta given that male Betta potentially incur substantial energy losses by establishing a territory for reproduction and vigorously defending the territory from intruding males, as well as, caring for fertilized eggs until the fry hatch.

*Betta*, are a member of the Anabantidae family and inhabit shallow pools of stagnant water in lower Asia. In their natural habitat, male Betta primarily feed at a constant rate on mosquito lava in the wild. Using little to no olfactory senses, Betta rely on the visual presentation of food. Betta first approach and then consume the food substance on the top or in the water by making a snapping motion, gulping the food into their mouth. In addition, when eating, Betta tend to darken in color (Hogan, 1961).

In two previous studies, Betta preference between food and mirror presentations was investigated (Hogan, 1974). Hogan found that Betta living in a T-maze preferred food rather than a mirror presentation, while Betta transferred from a holding tank preferred a mirror presentation rather than food. Researchers stated that transferring Betta from a holding tank to the T-maze might be the reason for observed differences in choice.
In a similar study, Bols and Hogan (1979) found that food was selected more than mirror presentations.

In order to address the aforementioned problems, the current study focused on the effects of two abstract cue based discriminative stimuli compared to a location based discriminative stimulus (e.g., left or right). The location based discriminative stimulus was proposed as the comparison group based on the number of studies with fish that have used such a discriminative stimulus preparation. The two abstract cue based discriminative stimuli, color (Bronstein, 1984; Hogan, 1974; Zerbolio & Wickstra, 1979, 1980) and bubbles (Wirth, Lattal & Hopko, 2003), were chosen based on previous use, although limited, with fish species.

Therefore, the purpose of the current experiment was twofold. The first purpose was to provide an empirical comparison of three discrimination methodologies proposed for use in choice preparations with male Siamese Fighting Fish (Betta splendens). The second purpose was to increase the literature regarding choice in relation to food reward amount in Siamese Fighting Fish (Betta splendens). In addition, the results from the present experiment should provide an analogous methodology for use with fish that allows a comparative analysis of choice with the body of choice research based largely on pigeons, rats, and primates.

Method

Subjects. The subjects (n = 35) were healthy, adult male Siamese Fighting Fish (Betta splendens) obtained from a local supplier. The subjects’ length averaged 6 cm and the fish were red or blue in color.
**Apparatus.** The apparatus was a T-maze similar to the apparatus used by Bols (1976). The T-maze consisted of a start/goal box (20 x 5 x 11 cm) and a swimway (40 x 11 cm). The T-maze was submerged in a tank (65 x 45 x 15 cm; approximately 30 L). Each tank consisted of a gravel floor, a temperature gauge, a submerged tank heater, an air stone, and a T-maze. All latency measures were obtained using a digital stopwatch.

**Procedure.** The water used in the apparatus was de-chlorinated before subjects were introduced and water temperature was regulated at 25° C throughout the experiment. Subjects were housed in the entire T-maze during the experiment and fed a diet consisting of a total of six Betta baby pellets (Hikari, Himeji Japan) daily; the pellets obtained during the task or supplemented after the daily session. Lighting was controlled on a 12-h:12-h light/dark cycle.

Subjects were randomly assigned to one of three discrimination groups. For all three Discrimination groups, subjects chose between one or three food pellets. In the Direction group, subjects relied only on left or right directions to discriminate between choices. In the Color Group, subjects were provided with a red or green (3M red and green tape, see Bronstien, 1986 for detailed description) discriminative stimulus. Red tape was placed such that the color surrounded one of the two entrance holes on the choice door, as well as, the same half of the choice door. Green tape was placed such that the color surrounded the opposite entrance hole in the choice door, as well as, the opposite half of the choice door. In the Bubble Group, a plastic tube with small punctures was placed in the goal box directly behind the choice door. This tubing was connected to an aerator and when activated, the bubbles were released behind one of the entrance holes in the choice door, while the opposite hole in the choice door had no bubbles present.
Therefore, when entering the goal box, the subject swam through bubbles, if choosing the side with the bubble discriminative stimulus.

To ensure that each subject experienced both choices, all subjects were exposed to four forced choice trials over two sessions (two trials per day). Forced choices were established by blocking one of the choice options, leaving the opposite unimpeded. The order of exposure to the reward options in the forced choice trials was counterbalanced to eliminate any potential primacy or recency effects. After the completion of the forced choice trials, subjects were tested for two free choice trials each day for a total of 14 trials. The side for presentation, as well as, the discriminative stimuli associated with the specific food option was counterbalanced across subjects to eliminate any potential bias.

Each subject's start box latency, swimway latency, and choice were recorded for each trial. Before the beginning of each trial, the subject swam into the start box and the start box guillotine door was inserted. Once the choice door was in place, the start box guillotine door was removed, initiating the trial and the start box latency measure. Immediately after the subject entered the swimway, the start box door was replaced, the start box latency measure ended, and the swimway latency measure began. Once the subject swam down the swimway and through the goal box choice door (choosing either one or three pellets), the swimway latency measure ended. The subject remained in the goal box until the food was consumed. Approximately 10 mins after the end of the trial, subjects were provided with any supplemental pellets needed to meet the daily requirement of six pellets.
Results

The total number of large reward choices were summed across the last five trials and divided by five to produce a proportion of large reward choices. A one-sample t-test was conducted on the proportion of large reward choices for each discrimination group, see Table 1. The Bubble group showed a significant choice preference for the large reward option ($t(11) = 3.26, p < .05, d = 0.94$) while choice preference for both the Direction and Color groups failed to deviate from chance performance ($t(10) = 1.14, p > .05, d = 0.18$ and $t(11) = 2.11, p > .06, d = 0.61$, respectively).

Table 1. Descriptive statistics for the proportion of large reward choices for each discrimination group.

<table>
<thead>
<tr>
<th>Discriminative Stimulus</th>
<th>Mean</th>
<th>s</th>
<th>n</th>
<th>Effect size (d)</th>
<th>Observed power (one-sample t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direction</td>
<td>.64</td>
<td>.40</td>
<td>11</td>
<td>.34</td>
<td>.18</td>
</tr>
<tr>
<td>Color</td>
<td>.67</td>
<td>.27</td>
<td>12</td>
<td>.61</td>
<td>.50</td>
</tr>
<tr>
<td>Bubble</td>
<td>.78</td>
<td>.30</td>
<td>12</td>
<td>.94</td>
<td>.84</td>
</tr>
</tbody>
</table>

Latency data were collapsed into 7, 2-trial blocks. Due to excessive outliers, Friedman’s ANOVA for ranks was used to analyze all latency data. Friedman’s ANOVA revealed a significant decrease in both start box and swimway latencies across the 7 blocks for the Bubble group (both $F_f$’s ($k = 7$) $> 18.43, p < .05$). Friedman’s ANOVA for both the Direction and Color groups failed to show a change in either start box or swimway latencies across the 7 blocks (all $F_f$’s ($k = 7$) $< 9.82, p > .05$).
Discussion

The present study indicates that subjects’ start box and swimway latencies decreased significantly over the 14 trials only for the Bubble group. Failure to find significant decreases in start box and swimway latencies in the Direction group and the Color group may be due to the small sample size relative to previous studies or the nature of the reinforcer. For example, in a study by Hogan (1974), 24 subjects, provided with a color discriminative stimulus (a black or white goal box), chose and swam faster for food than a mirror presentation. It is possible that by increasing the sample size in the Color group, results similar to that obtained by Hogan (1974) might have been observed. In addition to a significant decrease in latencies in the Bubble group, the present findings also revealed a statistically significant preference for the three pellets of food over one pellet of food only for the Bubble Group.

Of particular note is the effect size and observed power for each group. Clearly, the Bubble group is the only group which supports a reasonably powerful test for discrimination, given a sample size of 12 subjects and a 0.94 effect size. The tests for the Direction and Color groups produced smaller effect sizes and, consequently, are both underpowered, given their respective sample sizes, even though the Color group is on the cusp of statistical significance. A direct comparison of the mean differences and standard deviations shows that the smaller effect size for the Direction group resulted from a smaller mean difference coupled with a larger standard deviation. The larger effect size for the Color group, compared to the Direction group, is primarily due to a decrease in the standard deviation and secondarily due to a small increase in mean difference.
Finally, the larger effect size for the Bubble group compared to the Color group is exclusively due to the much larger mean difference.

Table 2. Sample size requirements for the discrimination groups across fours levels of power.

<table>
<thead>
<tr>
<th>Power</th>
<th>Discriminative Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direction</td>
</tr>
<tr>
<td>.60</td>
<td>42</td>
</tr>
<tr>
<td>.70</td>
<td>53</td>
</tr>
<tr>
<td>.80</td>
<td>67</td>
</tr>
<tr>
<td>.90</td>
<td>88</td>
</tr>
</tbody>
</table>

Table 2 presents the sample sizes required to achieve various levels of power for the three discrimination groups given the effect sizes observed for each group in the present experiment. Clearly, the sample sizes required to conduct an acceptably powerful test for the Direction group are exceedingly large compared to either the Color or Bubble groups. Moreover, the mean difference observed for this group does not present a strong choice bias, even if sufficiently powered and statistically significant. While the Color group requires far fewer subjects to conduct an acceptably powerful test, the number of subjects required is still nearly double that of the Bubble group to achieve an equally powerful test.

A review of common discriminative stimulus procedures used with Betta shows that most are based on direction or location (e.g. Bols 1976; Bols & Hogan, 1979; Hogan, 1961; Hogan, 1974). Such studies have examined the relative reinforcing qualities of a variety of stimuli including live conspecifics and nonconspecifics, mirror images, models of conspecifics, and inanimate objects. With respect to the aforementioned studies, it is
clear that choice procedures in which the reward options differ in quality or kind may result in better discrimination, and consequently larger effect sizes, using a directional discriminative stimulus procedure than that observed in the Direction group from the present experiment.

The implications for a comparative understanding of choice suggested by the present discrimination methodology are twofold. First, the present methodology will allow for the inclusion and direct comparison of Betta choice and potentially other fish, with the current body of choice research which is based largely on pigeons, rats, and primates using food rewards. Second, Betta represent an asymmetry in parental investment that is not typical for the subjects normally employed in choice research. Specifically, in Betta the male is solely responsible for care of offspring whereas in pigeons offspring care is equally split between the two parents and in mammals the female is typically the sole provider of such care. Thus, comparisons based on parental investment and potentially sex roles, may be instructive in advancing a more comprehensive comparative understanding of choice.

Experiment 2: Risk-sensitivity to food amount in male Siamese fighting fish, *Betta splendens*

Much like other small avian and mammalian organisms studied in risk-sensitivity experiments, fish have been shown to be affected by variations in reward options (i.e., Roche, Dravet, Bolyard & Rowland, 1998). For example, Jack Dempsey cichlids, bitterlings, and spined sticklebacks have been shown to shift from risk averse to risk prone strategies as the result of changes in energy budgets, thus exhibiting risk-sensitive behavior (e.g., Young, Clayton, & Barnard, 1990; Croy & Hughes, 1991).
Roche, Dravet, Bolyard and Rowland (1998), examined the choice bias in Jack Dempsey cichlids, *Chichlasoma octofasciatum*. A stable energy budget was determined by allowing subjects to freely feed, measuring the number of food pellets consumed per hour, and then manipulating their energy budget by controlling the number of pellets consumed prior to and during experimentation. Subjects maintained on a positive energy budget were given a choice between a constant food reward and a variable food reward in both a discrete-choice treatment and a free-choice treatment. Subjects preferred the constant food reward as opposed to the variable food reward in both the free-choice and discrete-choice treatments. Therefore, male Jack Dempsey cichlids responded in a risk averse manner when on a positive energy budget compared to a negative energy budget.

Young, Clayton, and Barnard (1990) revealed risk-sensitive behavior in male bitterlings (*Rhodeus sericus*) using a free choice design. Before the experiment began, one group of subjects was deprived of food for three hours, which maintained the subjects at or above the required energy budget (positive energy budget). Subjects with a positive energy budget choose a constant food reward (risk averse) as opposed to a variable food option, which yielded a mean of one pellet. In another group, subjects were deprived of food for 48 hours before the experiment began. Subjects with a negative energy budget chose the variable food reward (risk prone). Therefore, male bitterlings responded in accordance with predictions made by the Risk-sensitive Foraging Theory and the Daily Energy Budget rule.

Finally, in an experiment by Croy and Hughes (1991), spined sticklebacks (*Spinachia spinachia*) were deprived food for 24 hours before the beginning of the experiment. Subjects were then allowed to sample two food reward sites, one containing
a constant reward and the other site containing a variable reward. Researchers revealed that subjects responded differently toward food reward sites as the result of energy reserves. At the beginning of the sampling procedure, subjects with a negative energy budget chose to sample the variable food site as opposed to the constant food site. As subjects became satiated, choice of food site shifted to the constant food site. Therefore, male spined sticklebacks responded in accordance with the Risk-sensitive Foraging Theory and the Daily Energy Budget rule.

Siamese Fighting Fish, *Betta splendens* (Betta), are a member of the Anabantidae family and inhabit shallow pools of stagnant water in lower Asia. Male Betta primarily feed at a constant rate on mosquito lava in the wild, although most experimental studies have used an array of different types of food. Using little to no olfactory senses, Betta rely on the visual presentation of food. Betta first approach and then consume the food substance on the top or in the water by making a snapping motion, gulping the food into their mouth. In addition, when eating, Betta tend to darken in color (Hogan, 1961).

Researchers, studying male Betta, have rarely used food as a reinforcer or unconditioned stimulus in experiments. However, food has been successfully used as reinforcers in instrumental and operant conditioning procedures using a variety of related fish as subjects (e.g., Hollis, 1999; Suboski, 1988). Food has also been used as unconditioned stimuli in experiments using classical conditioning with related fish as subjects (Suboski, 1988).

In an experiment by Hogan (1961), researchers studied the effect of deprivation on operant responding in male Betta. In an operant conditioning experiment, Hogan (1961) revealed that the number of worms consumed during an experimental session
increased as a result of deprivation. Betta deprived for six hours consumed a mean of approximately eight worms. When deprived for a 12-hour period, Betta consumed a mean of 16 worms. In addition, the results indicate that Betta increased the number of operant responses.

In a similar experiment, Hogan, Keist, and Hutchings (1970) studied the effect of a fixed ratio schedule of food reinforcement using male Betta. Hogan, Keist, and Hutchings (1970) indicated that as the fixed ratio reinforcement increased, the number of operant responses by Betta increased, however the number of reinforcements remained constant. Hogan et al., (1970) stated that one could expect quite strongly that "responding would decrease as the number of worms per reinforcement increased," although researchers indicated that the operant response increased even though food reinforcement remained constant (p. 356). Similarly, Goldfish bar pressing increased when given the option of pressing a bar that returned small or large portions of food (Rozin & Mayer, 1961).

In previous studies, Betta preference between food and mirror presentations was investigated (Hogan, 1974). Hogan found that Betta living in a T-maze preferred food rather than a mirror presentation, while Betta transferred from a holding tank preferred a mirror presentation rather than food. Researchers stated that transferring Betta from a holding tank to the T-maze might be the reason for Betta choice. Although, in a similar study, Bols and Hogan (1979) found that food was selected more than mirror presentations.

To access risk-sensitivity, male Betta were given a choice between a variable and constant food option using a T-maze similar to that used by Bols (1976). In addition,
given the results from Experiment 1, the bubble discriminative stimulus was used to aid in discrimination. A negative energy budget was created by reducing the suggested number of pellet by approximately 75 percent. Betta Baby Pellets (Hikari, Himeji Japan) suggests that Betta receive seven to nine pellets daily. The negative energy budget was maintained by manipulating the amount of supplemental food pellets that subjects receive in addition to pellets received in daily trials. In light of the negative energy budget, subjects were expected to choose a variable food option (risk prone) at a rate significantly greater than chance.

Method

Subjects. The subjects (n=15) were healthy adult male Siamese Fighting Fish (Betta splendens) obtained from a local supplier. The naïve subjects’ length averaged 6 cm and the fish were red or blue in color.

Apparatus. The apparatus was a T-maze similar to the apparatus used in the experiment by Bols (1976). The T-maze (see Appendix 1 for diagram of T-maze apparatus) consisted of a start box (20 x 5 x 11 cm), goal box (20 x 5 x 11 cm), and a swimway (40 x 11 cm). The T-maze was submerged in a tank (65 x 45 x 15 cm; approximately 30 L). Each tank consisted of a gravel floor, a temperature gauge, a submerged tank heater, an air stone, and a T-maze. All latency measures were taken using a digital stopwatch.

Procedure. The water used in the apparatus was de-chlorinated before subjects were introduced and water temperatures were regulated at 25 °C throughout the experiment. Subjects were housed in the entire T-maze until the beginning of each trial. Subjects were fed Betta Baby Pellets (Hikari, Himeji Japan). Each subject received a total
of five pellets per day through those acquired in the T-maze and necessary supplements. The light cycle was maintained 12 h:12 h light/dark cycle.

The experiment consisted of two forced choice trials for two sessions (two trials per day). During the forced choice trials, subjects were forced to choose either the constant option or the variable option and then the alternate option on the subsequent trial. This allowed each subject exposure to both choice options. After the completion of four forced choice trials, the free choices trials were delivered over the following 16 days, one trial per day. During the free choice trials, subjects were allowed to choose from both food options. The experiment was terminated after 16 free choice trials.

The choice options consisted of either a variable or constant amount of food. The variable food option resulted in the delivery of either one \( p(1)=.5 \) or five \( p(5)=.5 \) food pellets. The constant food option resulted in the delivery of three \( p(3)=1.0 \) food pellets. The side for stimulus presentation was counterbalanced across subjects to eliminate any potential side bias.

At the beginning of each trial, after the subject freely swam into the start box, the start box guillotine door was put into place. Once the choice doors were in place, the start box guillotine door was raised, beginning the trial and the start box latency measure. Immediately after the subject entered the swimway, the start box door was replaced, the start box latency measure ended, and the swimway latency measure began. Once the subject swam down the swimway and through a goal box choice door, the swimway latency measure ended and the subject's choice was recorded.
Results

The total number of constant reward choices were summed across the last five trials and divided by five to produce a proportion of constant reward choices. A one-sample t-test was conducted on the proportion of constant reward choices, see Table 3. No significant difference was observed between the constant reward choices (t(14) = .25, p > .05, d = 0.06) and chance performance.

Latency data were collapsed into 8, 2-trial blocks. Due to excessive outliers, Friedman’s ANOVA for ranks was used to analyze all latency data. Friedman’s ANOVA revealed no significant decrease in either start box (F_r (k = 7) ≤ 5.96, p > .05) or swimway (F_r (k = 7) ≤ 6.09, p > .05) latencies across the 8 blocks.

Discussion

Subjects demonstrated no statistically significant preference for the variable option (risk prone) or the constant option food (risk averse). In addition, subject demonstrated no significant decrease in either start box or swimway latencies. Thus, in the current preparation, subjects were not sensitive to variability in food reinforcement amount. The aforementioned results do not deviate from chance performance and cannot be predicted or explained using risk-sensitive theories. Therefore, since Betta responded indifferently to the variable and constant food option, this experiment failed to reveal conclusive evidence to solely support any theory of risk-sensitivity.

According to risk-sensitivity (Smallwood, 1996), the current study could be best explained by assuming that Betta foraging strategies are not effected by variability in reward amount. Daily Energy Budget (DEB) states that as a forager reaches a negative energy budget, the organism should seek a reward with the greatest amount of return
foragers. However, Betta cannot be compared to the majority of studies that have examined the effects of variation in energy budget in organisms that continuously forage. The majority of studies that have examined the effects of variation in energy budget in organisms that continuously forage, have only studied organism that forage at a high rate. For example, Barnard and Brown (1985) manipulated the food intake of common shrews (Sorex araneus L.). Common shrews must achieve a high rate of caloric intake per hour or death becomes increasingly probably. Such foraging may not be necessary for Betta except in certain situations. Given that Betta do not require a high rate of caloric intake per hour, a subject might choose indifferently if the subject forages at a continuously low rate and if the subject’s energy budget is somewhere between a positive and negative energy budget. In other words, given that the subject is not in a state of caloric depravity or prosperity, one might expect some choices to be devoted to a variable food option (similar to a subject with a negative energy budget) and some choices to be devoted to a constant food option (similar to a subject with a positive energy budget).

In addition, Kacelnik and Bateson (1996) argued that the DEB rule fails to account for any energy expenditure directed toward behaviors other than foraging. For example, the DEB rule describes a static environment with no competing behaviors such that an organism required to care for young, defend a territory and compete for mates or other behaviors that would deplete energy reserves might not be accurately described. In other words, the DEB fails to account for behaviors in which Betta are frequently engaged and how such behaviors may affect choice. For example, male Betta devote much energy and time to developing a territory for the purpose of reproduction. Given that the male Betta in the current experiment were never exposed to competition and not...
required to expend the energy necessary to maintain a territory, subjects' choices for food patches may not have been of critical importance. However, in a situation when male Betta are exposed to competitors, sensitivity to variability in food amount may be of critical importance due to the increased energy requirement to maintain a territory. Choice in such a situation with male Betta may be similar to that observed by Barnard and Brown (1985) in common shrews where subjects forage continuously at a high rate.

According to Scalar Expectancy Theory (Kacelnik & Bateson, 1996), a forager's choice is based on the value assigned to a reward option and by the forager's record of events (events such as variability in reward amount) in which a desirable reward was received (Kacelnik & Bateson, 1996). This expectation and record of events is created according to Weber's Law which states that any noticeable difference in an event produces a record of the event (i.e. a Just Noticeable Difference). Given a Just Noticeable difference between choice options, a differential value should be associated with each option. Using Scalar Expectancy Theory (SET), the current results could be explained in terms of a lack of expectation or record of choice options due ultimately to a lack of a Just Noticeable Difference between the two choice options.

Finally, another plausible explanation for the aforementioned results could be that the current study was not sensitive enough to detect a choice bias in Betta. For example, one potential problem with the current experiment was uncertainty regarding energy budget. No empirical evidence exists regarding the amount of food required by Betta per day thus making the manipulation of energy budget somewhat speculative. Another potential problem was the discrepancy between the constant and variable. It may have
been that the difference between the constant and variable reinforcer was not large enough for Betta to recognize a substantial difference in amount.

Future studies need to be designed to develop a basic understanding of caloric requirements and reinforcement amounts/probabilities in male Betta. For example, a future study needs to be designed to develop an understanding of energy budget or daily caloric requirements in Betta. Such an understanding would allow researchers to accurately manipulate energy budget and, in turn, determine the effects of variability in choice in food amount. Another experiment designed to determine sensitivity variability in food amount, where choices yield different returns (as opposed to risk-sensitivity studies where choices yield equal returns), should be conducted to determine if Betta are sensitive to variability. In addition, an experiment examining the effects of competition on sensitivity to variability would be informative.

General Discussion

Results from the previous two experiments provide, first, a useful methodology for studying choice in Betta, where choices are between constant amounts of food. Second the current experiments offers insight into the responsiveness of Betta toward constant food sources. Finally, the current experiments provide information regarding choice bias in Betta, where choices between food amounts are variable.

Clearly, Experiment 1 outlines the choice bias of Betta when an appropriate discriminative stimulus is used and reinforcement amounts remain constant. However, when a more complex choice was required, a choice between a variable and constant amount of food, no choice bias was observed. Given the capacity for Betta to distinguish between one and three pellets in Experiment 1, one would expect subjects in Experiment
2 to display a choice bias. Two plausible explanations for differences in observed results from Experiment 1 and 2 will be discussed.

First, Betta may not be biologically prepared to be sensitive to variability in food amount in certain situations. In other word, such complex choices may be out of the purview of Betta. Betta may only have the capacity to develop associations with or are only sensitive to simple food choices that do not vary in amount. As previously discussed, Betta consume food at a constant rate and deficiencies in energy budget may only be encountered in certain situations. For example, Betta might be sensitive to variability in food amount only when other competing behaviors are required, such as competing for a territory.

Second, in Experiment 1, choice options were one or three pellets with the difference in options being two pellets. In Experiment 2 the variable option resulted in the delivery of one or five pellets of food and the constant option resulted in the delivery of three pellets. Similar to Experiment 1, the difference between the variable and constant options in Experiment 2 was two pellets. Despite the similarity between experiments in the difference between choice options, Experiment 2 may require a more complex or subtle discrimination.

Regardless of the use of the bubble discriminative stimulus, subjects in Experiment 2 may not have been able to distinguish between a variable and constant food option that only differed by two pellets. A seemingly easy method to further examine this potential problem would be to us a variable and constant food option that resulted in a larger or more noticeable difference in amount. However by doing so, the manipulation of subjects’ energy budget becomes more difficult. By increasing the number of pellets in
the variable option, the minimum number of pellets consumed by each fish increases, either through choosing the variable option or through supplements obtained after the trial. Such increases may result in an overall decrease responding as well as failure to obtain a negative energy budget.

Finally, despite the wealth of research devoted to studying risk-sensitive behavior using a food reinforcer, few studies have attempted to generalize risk-sensitive theory to other stimuli or non-food rewards (see Bednekoff, 1996 for review). Therefore, an experiment that addresses the generalizability of the predications made by risk-sensitivity would be beneficial. For example, the aggressive display in male Betta has been of great interest to researchers due to the unique interaction between subject and conspecific and the use of such aggressive displays as examples of the preparedness of Betta to inhabit a specific niche. By driving away other males with aggressive displays, the chances of obtaining a territory suitable for reproduction, as well as, attracting a female for reproduction increase for the defending male (Hogan, 1961; Robertson & Sale, 1974). Therefore, aggressive displays may be viewed as a pseudo-sexual or reproductive behavior. Previous research has revealed that such interactions between subjects and conspecifics have the potential to reinforce operant responding and choice behavior in instrumental responding (e.g., Bols, 1976; Hollis, 1984; Bols & Hogan, 1979; Craft, Velkey & Szalda-Petree, 2003). By studying the aggressive display in male Betta display, researchers could determine not only more about the reproductive behavior of Betta but the generality of risk-sensitivity as a theory to describe reproductive foraging behavior.
References


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Appendix I

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