THE EFFECTS OF AQUARIUM SIZE AND TEMPERATURE ON COLOR VIBRANCY, SIZE, AND PHYSICAL ACTIVITY IN *BETTA SPLENDENS*

A Report of a Senior Study

by

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ABSTRACT

The purpose of this study was to determine if tank size and water temperature had on effect on the overall health of *Betta splendens* and to provide useful insight for potential owners. This study tested the hypothesis that both had significant effects on fish health and should be considered when purchasing and constructing aquaria by placing fish in one of four aquaria types. Once per week for nine weeks the length of each fish was measured and photographs were taken for color analysis. One-minute videos were recorded four times during the study to analyze the behavioral effects of the experimental variables. Ammonium concentration, pH, and temperature were recorded twice per week, once before and once after weekly water changes. All fish in the 'small heated' aquaria group experienced adverse health. Movement was significantly reduced in the 'small unheated' aquaria ($p < 0.001$). These results indicate that small aquaria are not suitable for maintaining the health of *B. splendens*.

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CHAPTER I

INTRODUCTION

Betta splendens, more commonly known as Betta fish or Siamese fighting fish, have become one of the most popular novelty fish in the world. The name *Betta splendens* translates to "the splendid battler" (Monvises et al. 2009) or "brilliant warrior" (Lertpanich et al. 2007), describing the two main characteristics of *B. splendens:* their scale coloration and their aggressive nature. While they have always been valued for their aggressive nature in countries such as Thailand where the species originated, they have recently become commercially important and socially interesting in countries such as the United States and Australia, because of their vibrant color and behavior. Selective breeding for 'fighting fish' by humans has been part of the culture of southeast Asia for hundreds of years and only recently shifted to produce aesthetically pleasing colors and fin types for international commercial sale rather than sport. As of 2005, bettas were the second highest producing ornamental fish in both revenue and number of individuals produced for market (Monvises et al. 2009). These now domesticated fish tend to grow to 3-6 cm in length with long fanning finnage and display a wide variety of vivid colors (Lertpanich et al. 2007). In contrast, in wild or traditional fighting bettas this large finnage is far less desirable as it provides an opponent with an area that is easily damaged during a fight. From an adaptive evolution perspective, strong bodies and hard scales are more desirable than fin length and color (Monvises et al. 2009). Wild fish in the *Betta* genus are usually smaller than captive-bred fish, generally only reaching 2.5-3 cm in length with dull bodies, usually a brownish-green hue, with blue-green displays along fin rays which are pronounced during the breeding season. In general, males are typically larger, more colorful, and more aggressive than females of the genus (Lertpanich et al. 2007).

Bettas are in the suborder Anabantoidei, the labyrinth fish. This group is characterized by chambers in the caudal region of the head that are highly vascularized. This suborder includes popular ornamental fishes, such as bettas and gouramies (family Osphronemidae), which are native to the tropical regions of Asia (Pough et al. 2009). The vascularized chambers extract oxygen from the air that the fish 'gulps' in and exchanges it for waste gases (Pough et al. 2009). This adaptation is necessary in the fish's natural habitat on the edge of rice paddies where the dissolved oxygen content is low (Jaroensutansinee and Jaroensutansinee 2005). This type of habitat tends to consist of shallow water with dense vegetation and low pH. High temperature, around 31.8 °C, and large territory size were also noted for *B. splendens* (Jaroensutansinee and Jaroensutansinee 2005). Thus, due to their increasing popularity and hardiness, *B. splendens* are often housed in small aquariums at room temperature. It has been questioned if such housing is suitable for maintaining fish health, however no studies have been conducted.

Tank Size and Fish Health

Numerous fish-husbandry guides indicate that tank size alone does not determine the growth or eventual size of the fish kept within it (Appendix A). However, various factors relating to tank size and fish care do have an effect on the overall health, and therefore sometimes size, of the organism. Variables such as water pH, dissolved gases, ammonia, and other organisms sharing the water space all affect the water quality (See Table 1). Additionally, the regularity and frequency of water changes will dramatically affect the overall quality of the living space for the organism. These factors must be individually assessed before tying them in with tank size.

Although water pH varies, it must remain within certain parameters and be maintained in a narrow range for specific species to remain healthy. The blood of fishes and other vertebrates usually remains at a pH around 7.4 with very little margin for shifts in acidity (Wurts and Durborow 1992) (Albers, 1970). Because the blood vessels of fish are so close to, and often interacting with, the aquatic environment of the organism via the gills, the pH of the surrounding water has a significant impact on the pH of the blood. Fish, in general, thrive in water with a pH similar to that of normal blood: around 7.4. When water pH gets too high or low, around 10 and 5 respectively, death occurs. Usually, the most desirable range is 6.5-9 for maintaining fish health (Wurts and Durborow 1992).

Table 1: Variables that have significant impacts on water quality, general teleost requirements, and the effects of the variables on teleosts. These requirements vary between species.

Variable	General Teleost Requirement	Effect	Citations
pH	6.5-9	• Significant differences can cause changes in blood pH, leading to stress and death. • Low pH, less than 5.5, causes proton movement from the body to cease. • pH outside the general range results in toxic 'ammonia plasma levels' and 'reduced swimming abilities.'	(Wurts and Durborow 1992) (Rankin and Lin, 1993) (Rankin and Lin, 1993)
Dissolved CO_2	Varies; example: maximum of 20 mg of free CO_2 for trout, 25 mg maximum for carp.	• Can cause suffocation. • Can alter water pH. • Hypercapnia, or elevated carbon dioxide tension, coupled with hypoxia causes respiratory acidosis. This leads to a decrease in oxygen affinity and sometimes carrying capacity of the blood. • When not coupled, fish exposed to hypercapnia can recover by significantly increasing blood plasma and taking up bicarbonate in exchange for chloride.	(Wurts and Durborow 1992) (Svobodová, 1993) (Jensen et al.1993) (Jensen et al.1993)

Table 1 Continued

Variable	General Teleost Requirement	Effect	Citations
Dissolved O_2	Varies based on size and species.	• Concentrations of dissolved oxygen lower than the organism's requirement leads to suffocation. • Hypoxia, or oxygen deprivation, occurs when the oxygen tension is low. • Some fishes have developed organs to allow air breathing in hypoxic conditions, such as labyrinth organs.	(Jensen et al., 1993) (Pough et al. 2009)
Ammonia and other Nitrogenous Wastes	Maximum of 0.5 mg per liter in most teleosts.	• High concentrations of ammonia can cause necrosis of the gill tissues, increased energy use to detoxify ammonia, and tissue necrosis. • In elevated concentrations, nitrite iodizes haemoglobin to methaemoglobin, inhibiting the transport of oxygen.	(Jeney et al. 1991) (Jensen et al. 1993)
Live Plants	Not required but can have significant impacts	• Detoxify nitrogenous wastes, decrease CO_2 concentrations, increase O_2 concentrations.	(Gersberg et al. 1986)

Dissolved gases, namely carbon dioxide and oxygen, greatly influence other water factors and the environment's conduciveness for life. Carbon dioxide exists in water as a dissolved gas and as carbonic acid, both of which influence aquatic life at various levels. As carbonic acid, H_2CO_3, carbon dioxide can lower the pH of water (Svobodová, 1993). This lowering can lead to stressful conditions for fish and, eventually, death (Wurts and Durborow 1992). When an environment is high in free carbon dioxide the transfer of CO_2 from the blood to surrounding waters is greatly reduced, leading to acidosis. Some fish can adapt to this by adjusting blood bicarbonate levels, but when high CO_2 is coupled with low oxygen levels the result is often fatal for teleosts (Svobodová, 1993). Dissolved oxygen levels depend on input from photosynthetic processes and air and outputs such as fish and plankton respiration. Decreased oxygen concentrations, especially when combined with elevated carbon dioxide concentrations, readily leads to suffocation. The oxygen requirement differs based on the specimen's weight and species, but without additional organs, such as a labyrinth, the result of low oxygen content in water is death.

Because of the interaction between gill capillaries and the aquatic environment the blood chemistry of teleosts can be greatly altered by changes in water chemistry (Blaxhall, 2006). The detoxification of high ammonia concentrations results in changes in fishes. When free ammonia is in concentrations greater than 1000 $\mu g\ l^{-1}$ key enzymes in ammonia detoxification, namely glutamic acid dehydrogenase (GIDH), glutamic-acid-oxyl-acetic-acid-transaminase (GOT), and glutamic-acid-pyruvic-acid-transaminase (GPT), can cause a decrease in blood serum ATP (Jeney et al. 1991). These changes are also significantly affected by dissolved oxygen concentration, pH, and temperature (Jeney et al. 1991). This increased energy output to detoxify ammonia, leaves fish with less energy to put into other required functions. Additionally, high ammonia concentrations coupled with increased temperature and poor water quality tend to lead to tissue necrosis. This further increases the energy demands on the organism (Jeney et al. 1991).

Plants also play a major role in determining water quality in aquatic environments. For example, plants have an active role in increasing water pH via photosynthesis during the uptake of carbon dioxide. Carbonic acid is removed from water when CO_2 is taken up for photosynthesis, thus raising the pH. In low alkalinity environs where there is little buffering capacity this can cause dangerous fluctuations. However, in water with high alkalinity and thus high buffering capacity, the pH change is greatly reduced and stabilized (Wurts and Durborow, 1992). It has also been noted that plants are capable of taking up toxic nitrogenous wastes from the environment, namely ammonia, thus relieving other organisms, such as fish, from having to detoxify it themselves (Gersberg et al. 1986).

These factors; pH, dissolved gases, ammonia, and plants; are all affected by environment size. A smaller volume of water can reach toxic levels much more quickly than a larger volume because there is less water area for toxins to disperse into. This makes tank size a very important factor in fish health. Although these factors have significant impacts, another highly influential factor must be considered: temperature.

Water Temperature and Fish Health

The greatest difference between ectothermic and endothermic immune functions is the temperature of the environment. A review of these studies by Bly and Clem (1992) outlined the notion that “higher environmental temperatures … within the physiological range, enhance immune responses” and that “lower environmental temperatures (within the physiological range) tend to inhibit immune responses in ectotherms” (p. 159). The authors later discuss that the 'permissible' temperature range varies between different species of fish (Bly and Clem, 1992). Other studies found that increasing water temperature led to increased cardiovascular output coupled with decreased circulation time and no change in hematocrit or blood volume (Barron et al. 1987). Thus, higher temperatures within the optimal range of the species typically leads to healthier fish with stronger immune functions.

Water temperature outside the optimal range can have significant effects on growth and formation. A study conducted using seven-band grouper larvae *Epinephelus septemfasciatus* found that specimens reared at elevated temperatures grew faster than cohorts in cooler water, however survival rates were highest in the optimal temperature range of 25° and 26°C (Tsuji et al. 2014). A separate study demonstrated that the growth of brown trout *Salmo trutta* was significantly lower at fluctuating temperatures. When this was coupled with low water levels, researchers observed negative growth (Flodmark et al. 2004). This suggests that water temperature and stability are critical during teleost development and growth. This effect on growth, and therefore energy requirements, also affects behavior. With increased metabolic rates organisms may be more likely to take risks during foraging that they would otherwise avoid (Lienart et al.2014). Thus, exposure to significantly elevated temperature could cause increased casualties in a population, reducing the genetic diversity. When interacting with factors such as elevated ammonia and decreased oxygen, any negative effects of water temperature on growth will be augmented (Moyle and Cech, 128-129 p.).

In the context of *B. splendens* housed in aquariums temperature is not applicable to the concepts of predation and foraging, but it is clearly a vital facet to keeping healthy fish. Knowing, understanding, and providing the optimal temperature appears to play a key role in keeping fish growing properly and disease free.

Current Betta Husbandry Practices

People wishing to purchase *B. splendens* can find information on ‘proper’ care and husbandry practices from various resources, such as pet retailers and online enthusiasts. However, this information varies greatly between sources and is usually provided with little or no scientific evidence to justify it.

The care sheet provided by Petco, an international pet retailer, lists the minimum tank size required to house a single male *B. splendens* as “¼ +” gallon with a water temperature between 72 and 82°F (Petco, 2014). The retailer sells tanks specifically labeled as aquariums for bettas, such as the half-gallon Aqueon Betta Bowl Aquarium Kit and the half- or one-gallon Petco Glass Footed Betta Bowl (Appendix A). Other retailers suggest an aquarium that can hold at least two gallons of water, but market the same aforementioned half-gallon aquariums as ‘betta friendly.’

Additional resources for people interested in betta care are the websites maintained by people who classify themselves as betta enthusiasts. One such enthusiast is a blogger and writer, Adam Sivan. Numerous articles are published on his website, bettasmart.com, about ‘proper’ betta fish care and housing. He has strongly denounced products such as the “teddy tank” and “Betta Vase” on the grounds that they provide the fish with an ‘inappropriate home’ and can be ‘dangerous’ and deadly for the fish (Sivan, 2012). Sivan, and many other freelance writers, assert that betta fish must be kept in environments larger than what is usually advertised as ‘betta friendly’ by retailers, but even here opinions differ. The author of the website Betta Care 101 suggests that fish be housed in tanks that hold at least 2.5 gallons of water that is maintained between 76-82°F (Appendix B). Sivan recommends nothing less than 5 gallons of heated water for various reasons, such as ease of establishing a nitrogen cycle and stabilizing water conditions (Sivan, 2012). An article published in Tropical Fish Hobbyist Magazine echoed these claims, yet again asserting that larger heated tanks were healthier for *B. splendens* than unheated bowls (Appendix C). The author also echoes the claim that live plants are highly beneficial in aquariums, claiming that live plants remove wastes, particularly nitrogenous varieties, and help imitate the species' native rice paddies. The article ends by asserting that proper care and housing can increase the fish's lifespan, optimize finnage and color, and maximize health.

With so many differing views and standards circulating, it can be hard to decide which source or sources to trust. Does one listen to pet retailers and purchase a stylish compact aquarium sold inexpensively next to the fish, or does one adhere to the standards of various enthusiasts and purchase large aquariums that require a large amount of counter space? With no betta-specific scientific evidence to refute or support a claim, how can a prospective owner be confident that they are choosing the healthiest option for their fish? As *B. splendens* become more and more popular as pets and even as a mode of pest control in underdeveloped countries for controlling mosquito populations, a guide to husbandry practices grounded in science rather than opinion is required (De Oliveira Lima et al. 2010).

Previous Research and Objectives

The aforementioned studies indicate that teleosts generally thrive in captivity if the domestic environment is similar to the fish's natural environ. However, no research has been conducted to ascertain if this hold true for *B. splendens* and what options provide the species with environs similar to those of the rice paddies where the species originated.

The goal of this research is to determine if aquarium size and temperature have a significant impact on the health of *B. splendens.* By exposing groups of fish to one of four environment types, we hope to support or refute some of the various claims made by betta enthusiasts and pet retailers with scientific evidence. By filling gaps in the current knowledge of betta husbandry it is hoped that the minimum standards of living for keeping healthy fish can be clarified for prospective and current owners. The effects of tank size and environmental temperature will be determined on a small scale, which will hopefully initiate more specific research in the future that can lead to an even greater understanding of the requirements of captive *B. splendens*.

CHAPTER II

MATERIALS & METHODS

Specimens and experiment design

Twenty *Betta splendens* males were purchased from Petsmart (Alcoa, TN) and quarantined for nine days prior to experimentation. The fish were then randomly assigned to one of ten subdivided tanks at the end of the quarantine period.

Five 10-gallon aquariums and five 2.5-gallon aquariums were fitted with dividers, splitting them into ten 5-gallon tanks and ten 1.25-gallon tanks. The Sabic .063” Clear Lexan dividers were secured using Clear Dow Corning 732 Multi-Purpose Silicone Sealant, both obtained from Bell Helicopter (Piney Flats, TN), to maximize strength and prevent the passage of water between the separate tanks. These dividers allowed the fish to see into the other half of the divided tank; thus, each fish could clearly see other male *B. splendens*. Gravel and one plastic plant were placed in each half of the divided tank to give the fish a place to hide and therefore minimize stress. A Hydor Slim Heater was added to five of the 1.25-gallon tanks and a Tetra HT Submersible Aquarium Heater was added to five of the 5-gallon aquariums. Thus, there were five ‘small 1.25-gallon heated’ tanks, five ‘small 1.25-gallon unheated’ tanks, five ‘large 5-gallon heated’ tanks, and five ‘large 5-gallon unheated’ tanks (See Figure 1). Each tank was fitted with a clear lexan lid to prevent fish from jumping out of the tanks and prevent water loss due to evaporation.

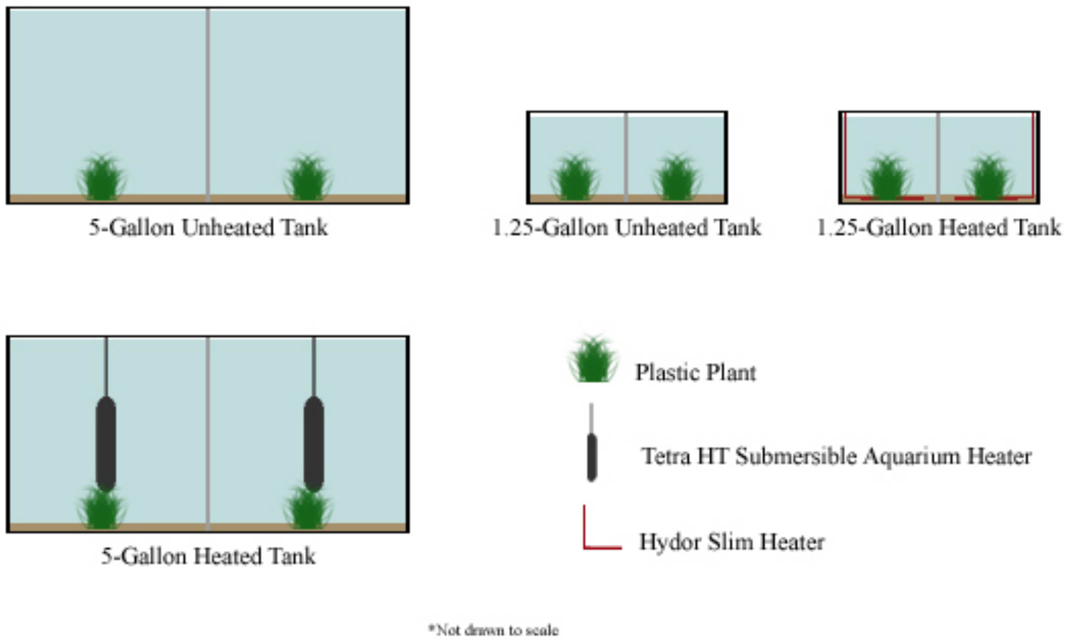


Figure 1: The experimental design for each group. Each 5-gallon tank group consisted of a 10-gallon aquarium divided into two 5-gallon segments. Each 1.25-gallon tank group consisted of a 2.5-gallon aquarium divided into two 1.25-gallon segments.

Specimen Care

Each fish was offered three (3) pellets of Aqueon Betta Food once per day. If the fish did not eat all of the pellets no additional food was given.

Half of the water in each tank was replaced with new water once each week, henceforth known as "water changes." Tap water was treated with Top Fin Water Conditioner according to the manufacturer's instructions in 5- or 3-gallon water bottles.

These bottles were left uncapped for a minimum of 24 hours to maximize treatment efficiency.

During the experiment a three week hiatus was required to treat a parasitic infection, *Ichthyophthirius multifiliis*. During this time no measurements were taken and all specimens were treated with “Super Ick Cure” from API according to the manufacturers’ instructions. After the treatment was complete all of the tanks received complete water changes to remove medication and dead parasites from the environment.

Endpoints Measured

Four endpoints were measured during the experiment: (1) morphometrics, (2) water chemistry, (3) activity level, and (4) color intensity. Each fish was measured and photographed prior to being placed in its assigned aquarium. This was done by placing a fish in a half-gallon aquarium with an affixed ruler and pushing it to the front of the tank using a piece of plexiglass. A photograph of the measurement was taken. This was then repeated on the same day of each week for six weeks. Measurements were not taken during the hiatus period but resumed from two additional consecutive weeks one the treatment was complete. In all, eight weeks of measurements were taken.

Ammonium, pH, and temperature measurements were taken twice per week using Vernir LabPro equipment. These measurements were taken one day prior to and one day after a water change. These measurements were not performed during the three week hiatus previously mentioned.

One-minute video segments of each fish were recorded to analyze activity levels. No stimulus, such as food, was offered before or during the recording. Two segments were recorded at the beginning of the experiment and two at the end. To avoid experimenter bias, three volunteers viewed each segment and recorded how long each fish was active during the recording. These three times were averaged, giving each fish a single time.

Photos of each fish were taken for color analysis when they were measured each week using a Canon ROS Rebel digital camera. Due to the iridescent nature of *B. splendens* scales quantitative analysis was not possible. For this reason, qualitative analysis was used. Three volunteers individually viewed photos taken of each fish at the beginning of the study beside the final photograph of the same fish. Each volunteer decided if the fish's color had become more or less vibrant, or if the fish had 'lost' or 'gained' color vibrancy, or had not changed during the study. If the color vibrancy had decreased the fish was rated on a scale of -1 to -5. If the vibrancy had increased the fish was rated on a scale of 1 to 5. If the fish's color had not changed the fish was given a 0 rating. The values from each volunteer were averaged to give a single value for each fish.

Data Analysis

One-way ANOVA tests conducted in SPSS were used to determine if there were any significant differences in water chemistry, morphometric changes, color vibrancy, and movement. If a significant difference was noted, a Tukey post-hoc analysis was used to identify these differences.

CHAPTER III

RESULTS

During the study four fish in the heated 1.25-gallon aquarium group died and thus were no longer part of the study post mortem. One of these casualties was due, presumably, to an *Ichthyophthirius multifiliis* infestation, and three to unknown cause. The only two fish affected by *I. multifiliis* were in the heated 1.25-gallon group. One fish in the 5-gallon unheated aquarium group had to be removed from the study due to severe injury obtained during a fight after a different fish jumped into his tank. This was the only incident involving a fish jumping over a tank divider.

Of pH (Figure 2), ammonium (Figure 3), movement (Figure 4), color (Figure 6), and body growth (Figure 6), with movement being the only variable significantly influenced by the experimental conditions ($p < 0.001$, see Table 2). Tukey post hoc analysis showed that the only group exhibiting this difference was the ‘Small Unheated Tank’ group, which significantly differed from the other three groups. Although there were numerous outliers, none were removed during analysis due to the small sample size.

After the analysis indicated that there was significantly less movement among fish in small unheated aquariums the raw data was once again analyzed and it was observed that one fish always tended to move more than his neighbor after the first week. For example, B19 and B20 were each other's closest "neighbor" during the study. During the first 1-minute recording, B19 and B20 were observed moving for 33.3 seconds and 31.9 seconds respectively. During the second video, these respective times decreased to 11.0 seconds and 16.0 seconds. From this point on, B20 consistently moves more during the 1 minute videos than B19, eventually achieving a maximum difference with 35.6 seconds (B20) and 9.5 seconds (B19). This trend was seen across all fish in small unheated tanks and their closest neighbors. One fish always appeared to take a dominant role, moving and flaring, while the other sook on a submissive role, remaining still without much fin display. This consistency of one moving more than the other was not observed in any other group.

Water chemistry data collected throughout the study are presented in Appendix 1. Temperature, ammonium, and pH values are shown. All photo plates used during color change analysis are presented in Appendix 2.

Table 2: One-way ANOVA results for each variable tested.

Variable	P-Value
Movement	< 0.001
pH (Water Chemistry)	0.354
NH^{+}_{4} (Water Chemistry)	0.508
Body Growth	0.481
Color Intensity	0.161

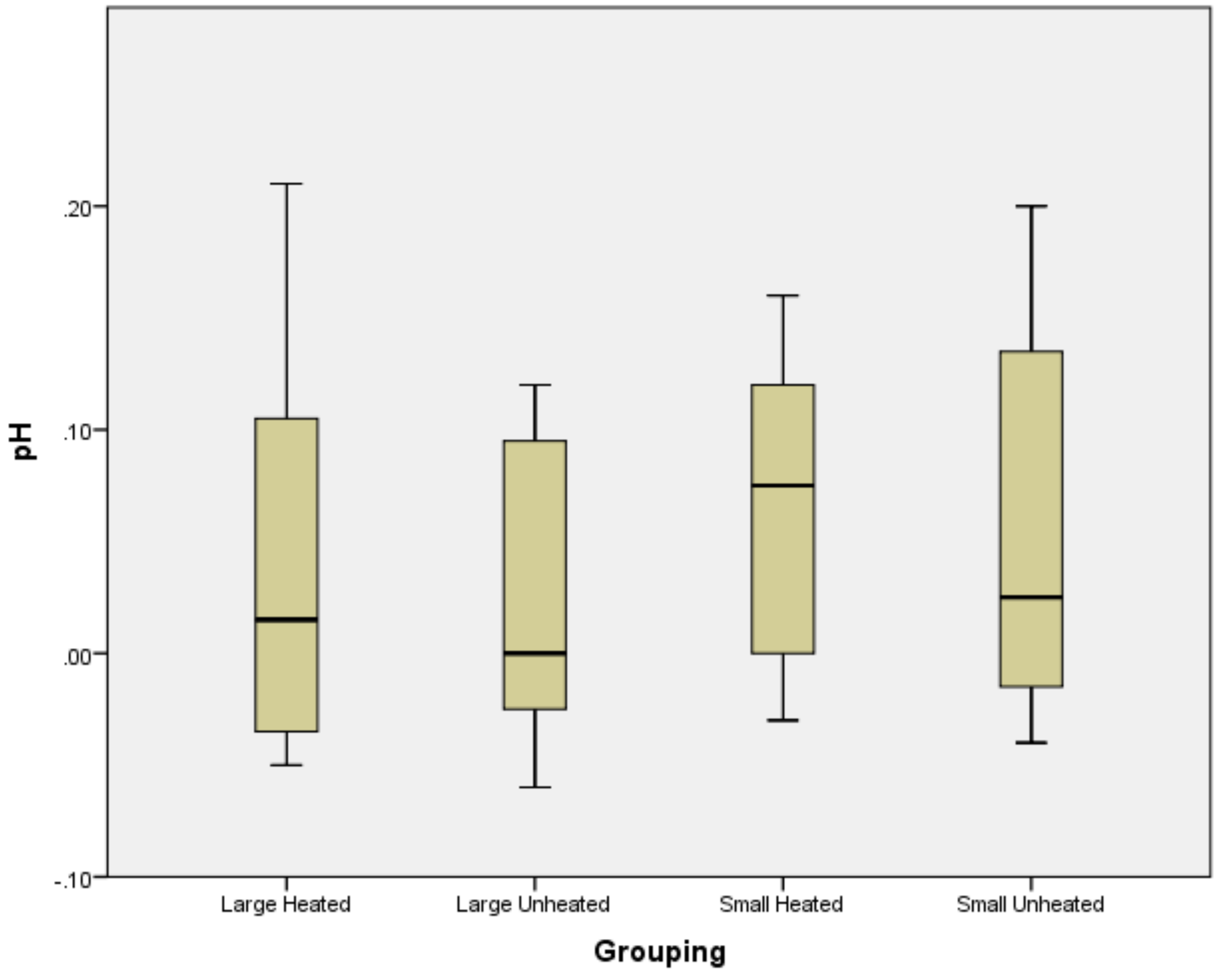


Figure 2: A boxplot displaying the distribution of pH changes among the four experimental groups between water changes. There was no significant difference among the groups ($p = 0.354$).

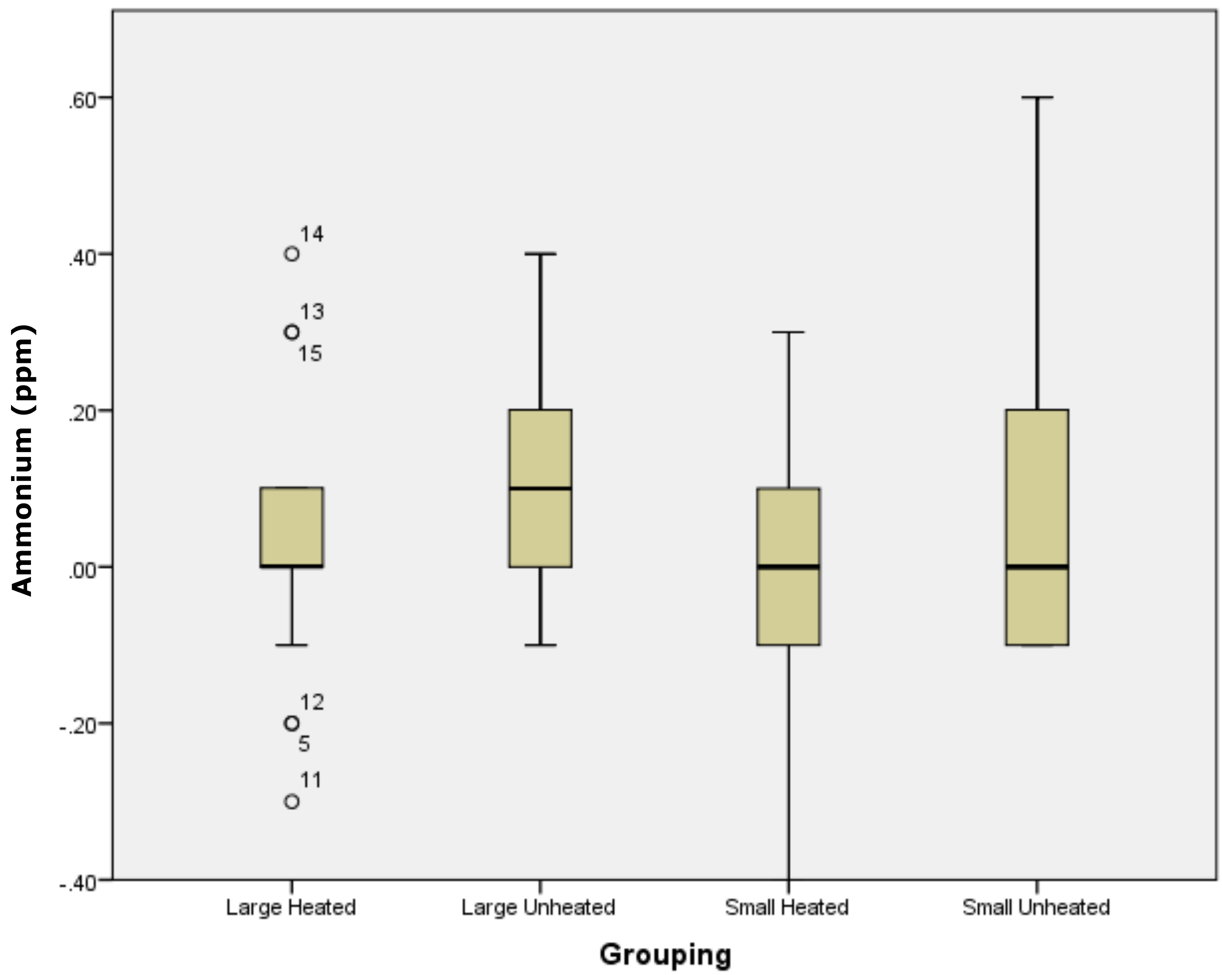


Figure 3: A boxplot displaying the distribution of ammonium changes among the four experimental groups between water changes. Data analysis revealed there to be no significant differences among the four groups (p = 0.508). Circles indicate an outlier more than one standard deviation from the mean.

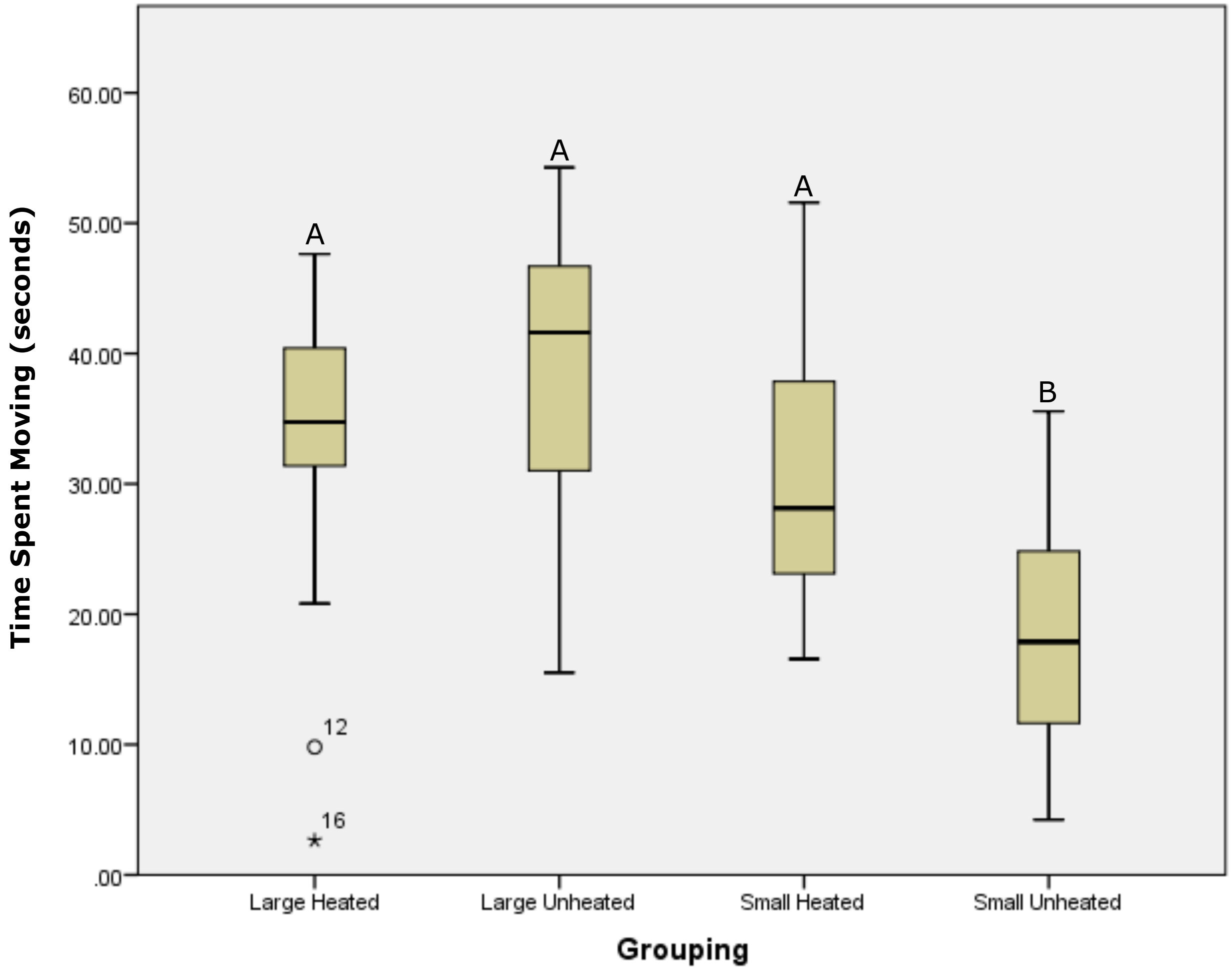


Figure 4: A boxplot displaying the differences in the amount of time specimens were active during a one minute period among the four experimental groups. Different letters above the bars indicate a significant difference ≤ 0.028. Circles indicate an outlier more than one standard deviation from the mean. An asterisk indicates an outlier more than two standard deviations from the mean.

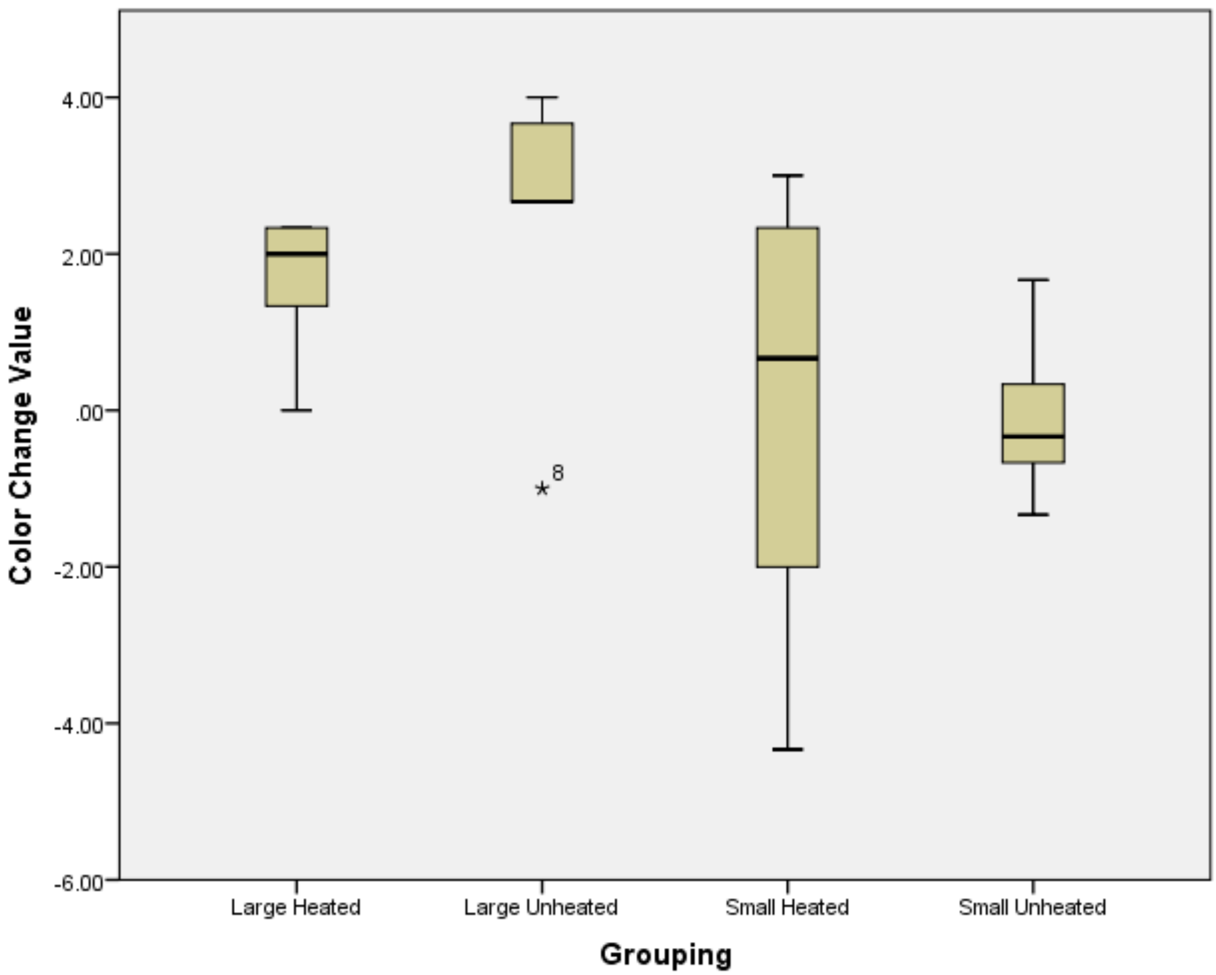


Figure 5: A boxplot displaying the differences in changes in color intensity during the experiment among groups. There was no significance among any of the groups ($p = 0.161$). An asterisk indicates an outlier more than two standard deviations from the mean.

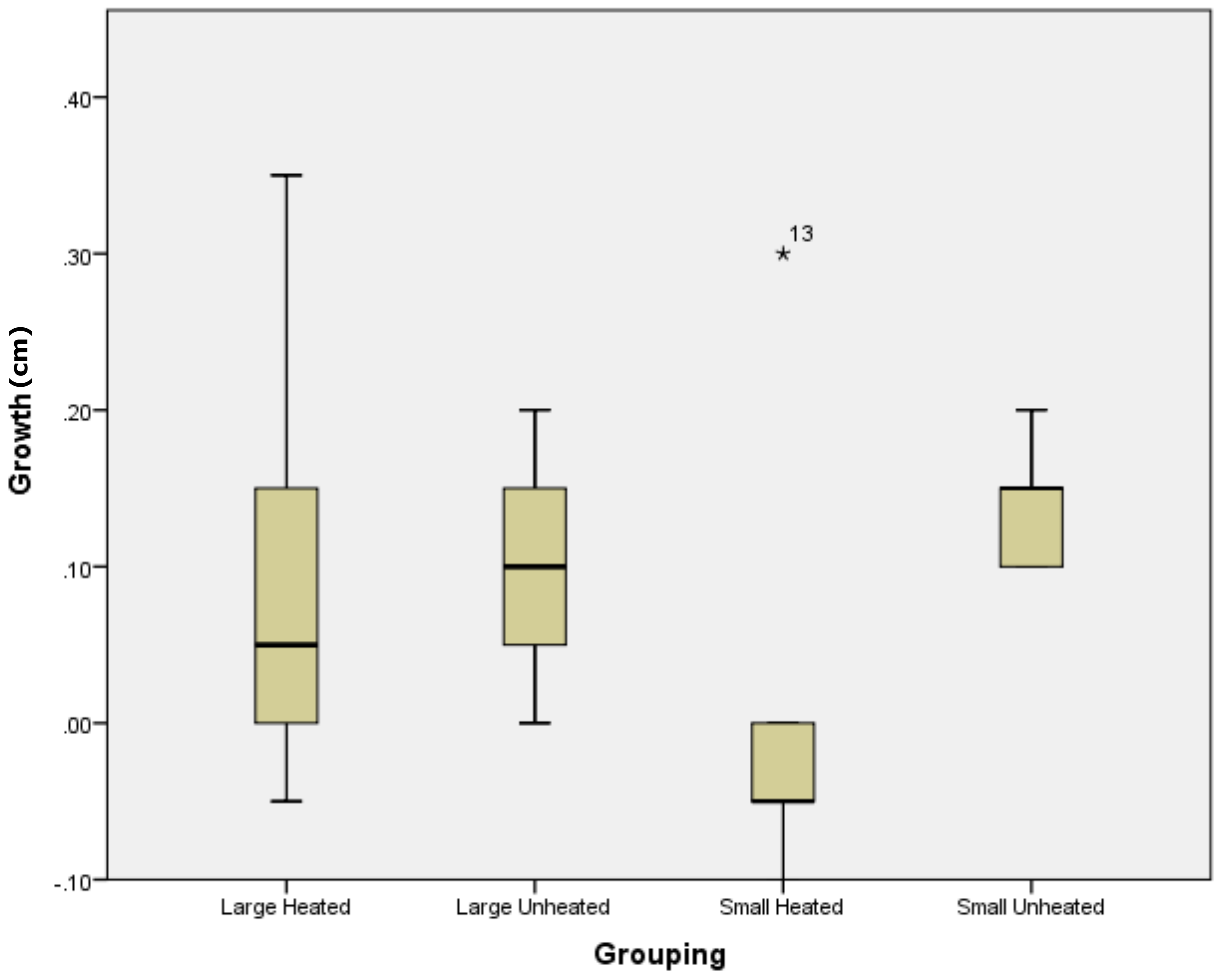


Figure 6: A boxplot displaying the differences in morphometric changes during the experiment among groups. There was no significance among any of the groups ($p = 0.481$). An asterisk indicates an outlier more than two standard deviations from the mean.

CHAPTER IV

DISCUSSION

This study found that *Betta splendens* movement was significantly reduced in the 'small unheated' aquaria compared to all other groups, indicating that both tank size and water temperature affect the overall health of these fish. No significant differences were found among the four experiment groups in any water chemistry factors, color vibrancy, or morphometric changes.

During data analysis, it was observed that one fish always seemed to move less than its closest neighbor, possibly indicating a 'dominant-submissive' relationship. This trend could be partially explained by the idea that a smaller area and fewer perceived resources, leads to increased aggression and the establishment of a hierarchy. Among betta fish, physical space has been shown to be inversely related to attack incidence, but not displays (Oldfield 2011). Similarly, a relationship between higher habitat complexity and reduced aggression was also observed using cichlids (Oldfield 2011). In the case of a complex environment it was observed that there was usually not a single fish dominating the space. However, when the environment was simplistic or object sparse there was usually one fish that dominated the rest of the school. In zebra fish (*Danio rerio*), monopolization of food resources in the group and aggressive behavior by the dominant fish were both significantly decreased when complexity was added to the environment, but increased when the aquariawas made simple (Basquill and Grant 1998). In the present study, *B. splendens* were provided only aquarium gravel and a single plastic plant. It is possible that the small area coupled with the lack of environmental complexity could have contributed to the development of a dominant-subordinate dynamic between neighbors. This could, in part, account for the difference.

It was hypothesized that color vibrancy would be affected by tank size and water temperature, but no statistical differences were observed among the groups; however, individual color changes, sometimes drastic, were observed. Although the effects of temperature on the color of *B. splendens* has not specifically been studied, it has been studied in other fishes. For example, elevated water temperature caused "melanosome aggregation … in melanophores" in trout. However, in *Phoxinus* this aggregation was observed when cool water was applied and dispersion was noted when warm water was applied (Fujii 1969). This suggests that temperature effects the color of each species differently.

Another interesting trend was the tendency of fish in the small heated tank group to experience adverse health effects. Of the five original fish assigned to this group, only one survived the study. This was the only group to experience spontaneous death and disease. Although all of the fish in the experiment were medically treated for *I. multifiliis,* the only individuals to exhibit symptoms were B16 and B17. B16 did not survive the infection. B17 survived the experiment, but later had to be treated for a second 'ich' infection.

Deaths during the study were only observed in small heated tanks. Because temperature was the only variable that differed among the group that experienced fish death and those that did not it can be suspected that there is some correlation between temperature and death/increased susceptibility to parasitic infestation. The tendency for fish in the small heated tanks to develop ‘ich’ can be partially explained by understanding the parasite’s growth. Aihua and Buchmann (2001) observed that the escape of theronts from tomocytes decreased from 8-9 days to 16-27 hours at temperatures of 25°C and 30°C. The large heated aquaria tended to stay no more than 0.5°C above or below 25°C, however the small heated tanks tended had a wider range, usually between 28.0°C and 28.9°C, but occasionally cooling to around 26°C. It is possible that this temperature difference was related to the lack of ‘ich’ symptoms in fish outside the heated aquaria group, but not the lack of symptoms in the large heated tank group. However, the lack of observed ‘ick’ in large heated tanks could be explained by the elevated temperature. Elevated temperature results in increased cardiac output (Barron et al. 1987). The temperature increase beyond the normal of *B. splendens* and resulting elevated cardiac output could have resulted in increased physiological stress. Although it cannot be confirmed, this alone or in combination with a rapid increase in parasite populations may have resulted in death of an individual.

Numerous studies examining the effects of temperature on health and survival rates point to a common trend: water temperature can significantly help or hinder an individual’s ability to survive. For example, the survival rates in juvenile *Thalassoma bifasciatum* tends to increase in warm water as growth rates accelerate (Grorud-Colvert and Sponaugle 2010). However, elevated temperature in also correlated with an increase in ammonia excretion in farmed palmetto bass (*Morone saxatilis x M. chrysops*). This excretion increase was also noted as animal size and population density increased (Liu et al. 2009).

Despite the wide array of research pertaining to temperature and its effects on fish, very little has been done to examine the effects of environment or aquarium size. Although some studies in aquaculture have found a correlation between high stocking density and higher rates of mortality and reduced growth in tilapia, the concept of stocking density is not an equivalent comparison to the concept of the required environment size for a single organism (Yi et al.1996). Future studies could address this by examining the health of solitary fish, such as *B. splendens*, in different aquaria sizes without altering other variables. Additionally, specific attention could be allotted to the effects of elevated temperature on betta color as an indicator of overall health. Although both of these were examined in the present study, the small sample size of each group and, thus, the inability to remove outliers, it is possible that significance was not found even when present. By testing variables individually with much larger sample sizes could provide additional data to support the null or clarify whether the outliers noted in the present study were truly outliers or within the normal range.

The purpose of the present study was to analyze some of the suggestions for housing *B. splendens*, tank size and the use of a heater, made by various sources. Overall, the data collected in the present study tend to support the use of larger aquaria while housing *Betta splendens* but was inconclusive about the use or disuse of a water heater. The significant difference in the amount of movement among the 'small unheated' aquaria with all others combined with the consistently reduced health and increased mortality rate in 'small heated' aquaria suggests that 'small' aquaria are not conducive to maintaining the health of the animal. It was unclear whether the addition of a heater to the larger tanks had an effect on fish health due to the small sample size and high occurrence of outliers in the majority of the data sets. Based on these findings, the recommendation to potential *B. splendens* owners and husbandry educators would be to avoid aquaria with a volume of less than five gallons and to attempt to provide a water temperature as close to that of the fish's natural habitat as possible. Figure 7 shows an example of an aquarium that would be suitable for housing betta fish based on the data collected and observed behaviors. Overall recommendations are outlined in Table 3.

Figure 7: An example of a suitable aquarium for *B. splendens.* Plants and rock décor provide complexity to reduce stress and aggression in a 5.5-gallon aquarium. A heater was provided due to the low temperature of the home to maintain a stable, healthy temperature.

Table 3: Recommendations for constructing a suitable aquarium for a *B. splendens* to maintain a healthy fish.

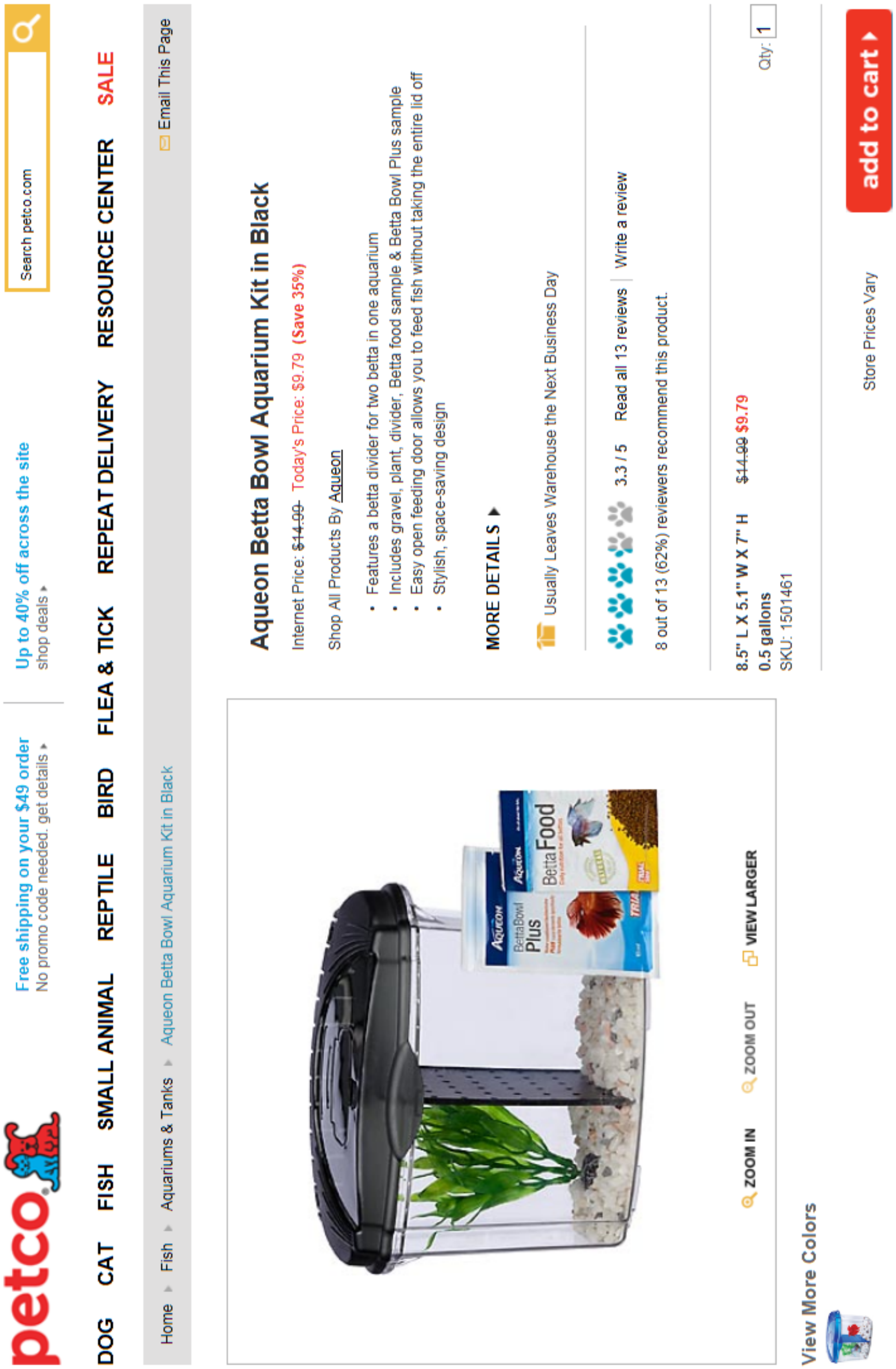
Variable	Recommendation
Size of aquarium	No less than 5 gallons
Use of a heater	It is best to try to maintain a water temperature close to that of the animal's natural habitat. The use of a heater is at the owner's discretion. General air temperature in the home should be taken into account.
Use of plants, décor, etc.	Create a complex, stimulating environment using betta-safe décor.

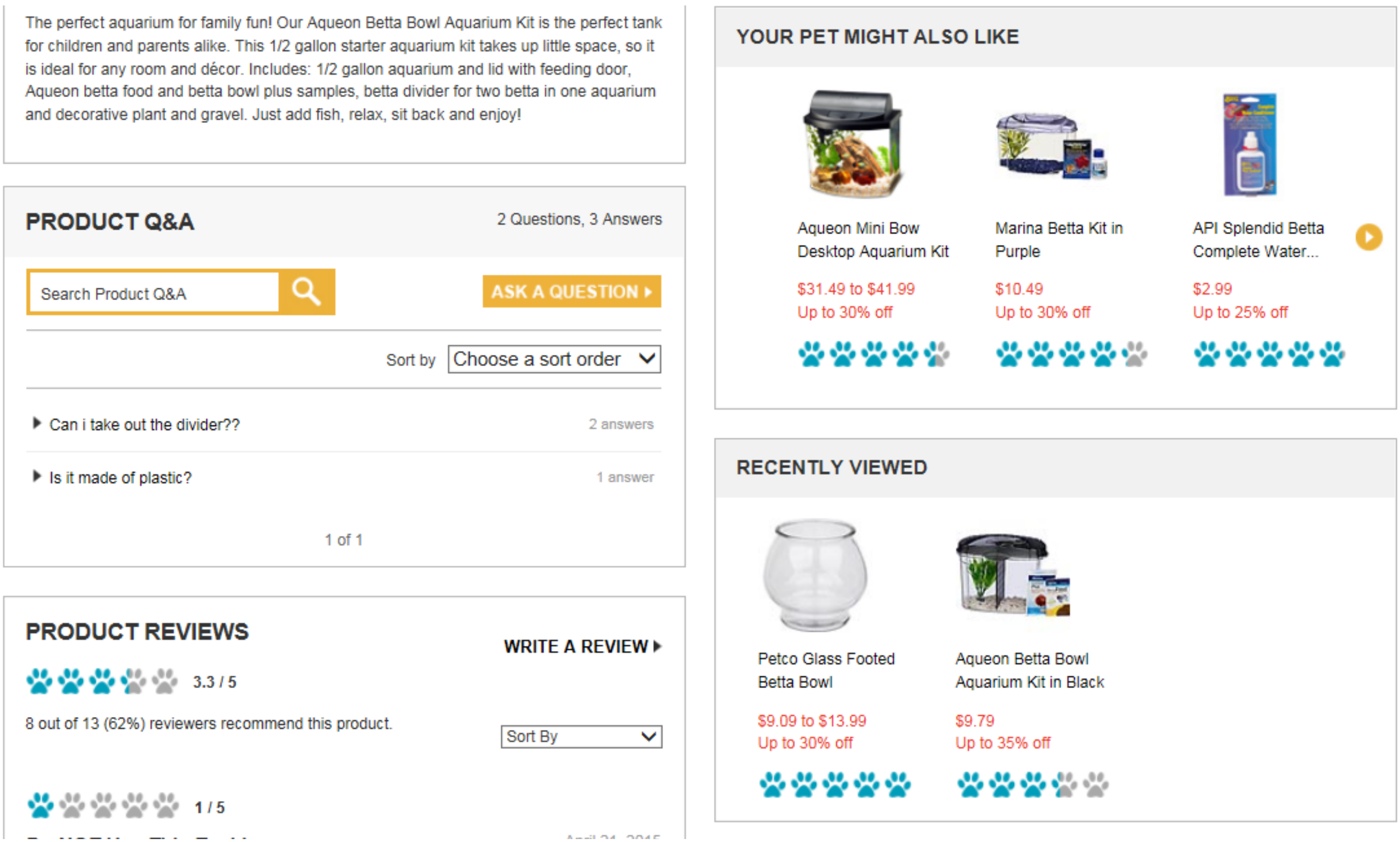
APPENDECIES

APPENDIX A

From:

http://www.petco.com/product/119418/Aqueon-Betta-Bowl-Aquarium-Kit-in-Black.aspx?CoreCat=MM_FishSupplies_FishTanksAquariums

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2 Questions, 3 Answers
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Choose a sort order
Can i take out the divider??
2 answers
Is it made of plastic?
1 answer
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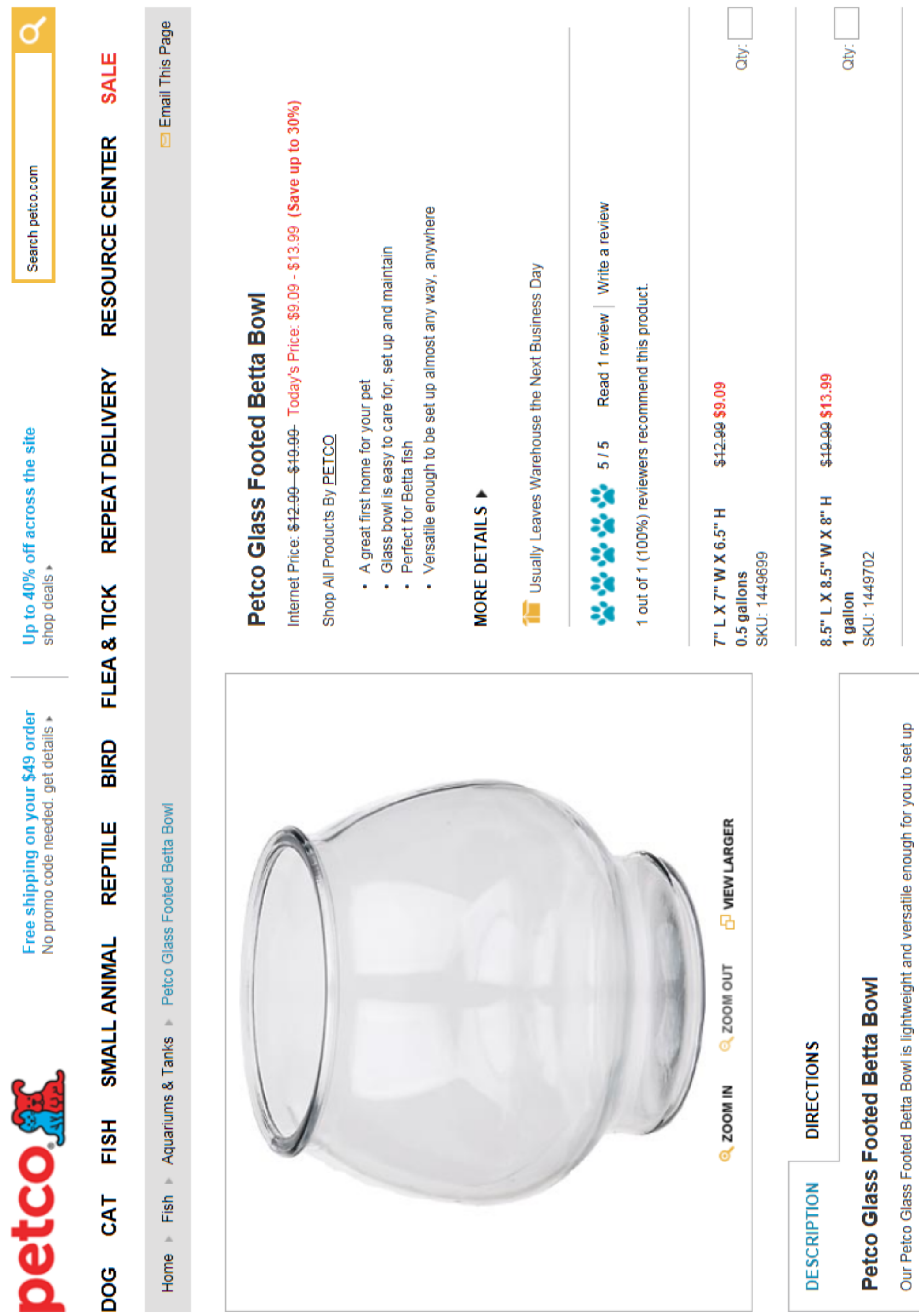
From:

http://www.petco.com/product/121350/Petco-Glass-Footed-Betta-Bowl.aspx?CoreCat=BettaHPFishTanks



Our Petco Glass Footed Betta Bowl is lightweight and versatile enough for you to set up practically any way and anywhere you please. This clear glass footed style bowl has been specially designed to house and to showcase your Betta fish, goldfish or other species of small freshwater fish. This attractive and easy-to-maintain fish bowl is great for both beginners and seasoned aquarists. This deluxe Betta bowl provides you with a 360-degree view of its aquatic inhabitants and fits right in with any home décor. Simply add some water, a plant or two, aquatic decorations, marbles or colorful substrate, along with a Betta fish or goldfish to gracefully swim around, and you can bring the beauty and serenity of an aquatic habitat to practically any room in your home.

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Betta

February 27, 2015

Dkravtsova1 from Rocklin, CA

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$9.79

APPENDIX B

From:

http://bettacare101.com/mythvsreality2/

Myth: Betta-in-a-vase products mimic the betta's natural environment and create a stable internal ecosystem
Reality: One of the most dangerous elements of the betta fad is the betta-in-a-vase craze. These vases are undersized, over-exposed, fail to maintain adequate temperature, and often do not provide space for air-breathing. It should be easy to see through the suggestion that they mimic the betta's natural environment; what natural environment could a tall, narrow container half-filled with marbles with a plant plopped on top possibly be emulating? Certainly not a rice paddy, that's for sure! What's more, the assertion that the plants roots will sufficiently manage ammonia and waste production in the small container is preposterous; even in aquariums filled with aquatic plants, filtration and gravel cleaning are required.

Myth: Bettas kept in vases survive by eating the plant's roots; feeding and cleaning is not needed since this is a self-sustaining environment.
Reality: Bettas are carnivores (insectivores) by nature; in the wild, they eat a variety of insects, and occasionally the eggs and fry of other aquatic species. While they might will intermittently nibble at plant matter if hungry enough, they can not obtain adequate nutrition from plants. Any betta actively consuming the roots of a plant is likely doing so as a last resort while attempting to stave off starvation. They absolutely must be fed an appropriate carnivore diet. Furthermore, as mentioned above, the vase is not a self-sustaining environment. True, a starved betta will not produce as much waste as a well fed one, but bettas absolutely need water changes - especially when kept in inadequately sized containers like a vase.

Myth: Bettas, even in small and unfiltered tanks, do not require frequent water changes as they thrive in unclean conditions.
Reality: Tying back to the myth regarding the ideal water conditions of bettas, it is a common misconception that the species is found in dirty, muddy puddles, which has convinced some novice aquarists that clean water is not demanded of the species and could even be harmful. In reality, the opposite could not be more true. The selection for decorative and show finnage types in bettas has created fish that are in fact highly sensitive to water quality; unless conditions are pristine (right down to hardness and pH, even!), you can expect crown tails, half moons, and other long finned bettas to suffer deterioration of the finnage. The poor circulation to the extremities of these lofty fins also makes them a prime target for bacterial infections, a problem only exacerbated by unclean water. Make no

target for bacterial infections, a problem only exacerbated by unclean water. Make no mistake: there is no such thing as a fish that thrives in waste-laden, filthy water!

Myth: Bettas do not require heating or filtration.
Reality: While this is technically a myth, there is no simple counterpoint for a number of reasons.It is true that bettas can survive without a heater or filter, but only under highly specific conditions. For example, in a room heated to at least 76 degrees, a betta does not need an actual heater, but under normal room temperature conditions, they absolutely do. The ideal range of the species is 76-82 degrees (with a survival range of around 72-86), which under most conditions demands a heater. In regards to filtration, opinions are split. The benefits of a cycled, filtered tank are numerous and well supported. Unfortunately, the combination of yielding from stagnant water and having large, cumbersome fins can make filtration stressful, so many keepers (including traditional thai breeders) do not filter or cycle. Both methods seem to work so long as clean water and consistent water parameters are maintained.

Myth: Bettas are not tropical fish.
Reality: Many pet stores will tell customers that cold cups are not problematic because bettas are not tropical fish. I'm sure the people of Thailand would be interested to learn that they are living in a temperate climate! Bettas, like many other tropical fish, have an ideal temperature range of 76-82 degrees. They survive perfectly well in temperatures upwards of 86 degrees, but they can not thrive in temperatures below 72. While they may be hardy enough to survive outside of their ideal range, cool temps typically result in lethargy, constipation, fin rot, and an increased susceptibility to disease.

Page: 1 2 3 4 5

APPENDIX C

From:

http://www.tfhmagazine.com/details/articles/better-bettakeeping.htm

Better Betta-Keeping

Author: Philip A. Purser

While many are content with keeping their betta in a small unheated bowl, these beautiful fish look better and live healthier lives in proper setups.

Photographer: Tony Terceira

"Excuse me, sir," a tiny voice called from behind me as I flailed about in another tank, fruitlessly chasing a specific Chinese algae eater with a net. Frustrated with the futile chase, I dropped the net into the aquarium and turned to face my questioner, a wide-eyed, blonde-haired boy, who couldn't have been more than 10 years old. In one hand he held a tattered slip of paper, and in the other fist he clutched a crumpled five-dollar bill.

"Yes?" I replied, wiping the aquarium water from my arms on a nearby utility towel. "What can I do for you?" The boy glanced down at the sheet of paper in his hand and cleared his throat. He twisted the toe of his shoe into the tile floor as he spoke.

“My daddy wants me to buy a…a…” His brow furrowed as he read the paper. “A betta *splendid*.”

I smiled and reached for another dipnet.
“A betta splendid, eh?” I joked as I headed for the betta display at the other end of the store. “I think we’ve got a few of those. What color does your daddy want?” The boy followed close on my heels and, after surveying each of several 5-gallon tanks in which our bettas were individually housed, he pointed to the most splendid pastel blue betta in the house.

The fish that the young man purchased was a *Betta splendens*, more commonly known as simply the “betta” or “Siamese fighting fish.” Frequently kept by hobbyists in tiny bowls, glass vases, or other desktop aquaria, the betta has long been a subject of controversy. Adherents on one side of the war say that bettas don’t need excessive space to swim and move, and that they are perfectly at home in a softball-sized bowl. Members of the opposite faction contend that no self-respecting aquarist would imprison an animal within such a minuscule enclosure. As a former fish retailer and longtime hobbyist who’s answered the same betta questions from customers more times than I care to remember, I’ve reached the conclusion that both sides of the betta argument are right and both are wrong. So dive in, if you will, to the underwater world of the bettas, and let’s answer once and for all some of those prickly questions about our flamboyantly finned friends.

Some Background
Understanding the biology and natural history of *Betta splendens* is paramount to really knowing your fish. Hailing from the warm, oxygen-poor waters of Thailand, Burma, and surrounding areas, bettas belong to a group of fish known as labyrinth fishes, whose name comes from a breathing-accessory organ known as a labyrinth. Functioning as a sort of rudimentary lung, this organ allows the fish to gulp air directly from the atmosphere; the fish need only break the surface with its mouth, swallow some air, and slip back under the water while the atmospheric air absorbs into its bloodstream. While not an overly efficient means of breathing, this method of augmented respiration is highly beneficial to bettas. In their natural environment of slow-moving, oxygen-poor waters, this adaptation allows them to live and thrive, despite such harsh conditions.

So a shallow tank of unmoving water is nothing new to a captive betta, and these fish have shown they can survive in a small bowl, but does this mean they can thrive in this type of environment?

Bigger Is Better
Speaking to the technical side of the matter, we know that more room to move about isbetter. A higher volume of water allows for more stable water conditions and thwarts the waste concentrations that a polluted betta bowl is subject to—it can go from livable to toxic literally overnight. A larger tank also grants the fish more room to move and swim about.

Having room to move about is critical, as recent scientific findings suggest that the long-term health of bettas housed in tiny enclosures is compromised, with the life span of closely confined specimens being drastically shorter than those animals that have plenty of room to swim about. Autopsied specimens that were kept in small enclosures have been found to have died from atrophied muscles and fatty degeneration of tissues, while their spacious-dwelling counterparts maintain a high degree of muscle tissue and experience *much longer* life spans. Specimens confined to tiny bowls seldom exceed 18 months to 2 years in captivity, while free-ranging specimens housed in larger aquaria may thrive for more than seven or eight years!

So larger is better in terms of tank size and the amount of space your betta has in which to live, but what type of larger aquarium is fit to adequately house a betta? Can the average hobbyist simply drop a betta into his or her tank without making any modifications catering to the betta's specific needs?

Let's look at the morphology of the betta in order to better understand this issue. Bettas have thin flowing fins that are very elegant and frail, even in the wild-type, short-finned varieties. These are fit for life in still water, though they are poorly equipped for rapid swimming in an area of swift current. Likewise, the gills of the betta are thick and full of filtering membranes, a sign that these fish are well adapted to life in silt-rich, oxygen-poor, murky waters.

Taken together, these adaptations, along with the presence of the labyrinth organ, allow for a fish that is suited for life in quiet, shallow, and muddy waters, as anyone who has ever captured wild bettas from their native swamps and rice paddies in southeastern Asia will attest. It is just that shallow, warm, still water environment that has supported bettas for millions of years. With this in mind, why would a hobbyist drop a betta in a deep, clear aquarium outfitted with powerheads and swift-moving filtration? While rigid-finned fish such as cyprinids, tetras, and barbs are well equipped for life in moving waters, bettas are easily overwhelmed by swift water currents. It is even worse for males of ornamental strains that have extra-long finnage. What some hobbyists might see as the generous act of granting freedom and more space by placing their betta in a standard aquarium might actually be doing a great disservice to an animal that is poorly suited to life under such conditions.

Optimal Housing

So an optimal betta enclosure must maintain a balance of space and sufficiently mild current. Excessively powerful and jet-like powerheads or other mechanical filters that severely roil the water have no place in a betta aquarium, as these items generate oppressive currents that will force your betta to seek out and remain confined to only the most placid, calmest sections of the aquarium. Aside from being physically oppressive, excessively powerful water currents are also a great stress factor on bettas. Continued buffeting and blustering by powerful currents can weaken a betta's protective slime coating and wear down its immune system in a short amount of time, thereby opening the door for bacteria and disease to take hold.

The alternative is a large community tank with minimal current. Employing a bio-wheel or trickle-style filter is a good start. Use a model that isn't too powerful, but which cycles at least five times the volume of the tank per hour, and extend the intake tube as deeply into the aquarium as it will go. Because bettas favor the upper reaches of the water column, they will not likely venture close to a deeply-positioned intake stem, so they won't have to fight the suction of the intake.

If possible, situate the return of this filter in such a way as to angle the returning water into one corner of the tank, leaving the rest of the aquarium largely current-free. Air-powered filters such as the undergravel or sponge filters are also great, since they do not produce much current and the air flow can be adjusted.

Living plants are great natural filters. Looking again at the betta's native environment, we see that the ponds and swamps of southeastern Asia that the fish calls home are usually choked with living aquatic plants. In the aquarium these plants absorb copious amounts of nitrogenous wastes from the water. Bear in mind, though, that many live plants may also require higher intensities of light than your tank may currently be receiving.

Denying your betta the benefits of filtration—as a great many hobbyists unwittingly do by housing their pets in desktop bowls and decorative glass dishes—will, in time, lead to health degradation and a shorter life span in your fish. On a purely aesthetic level, high levels of ammonia/nitrogenous wastes also cause a betta's fins to split, break down, and literally fall apart, leaving your poor fish pale, tattered, and stripped of its former beauty. If unchecked by biological filtration methods, these nitrogenous wastes will soon spike to lethal levels in an unfiltered bowl and claim your betta's life.

A Native of the Tropics

Another topic of controversy among betta keepers is the matter of temperature. Many hobbyists feel that housing their bettas at room temperature is a viable practice. This is not true. But who can blame the hobbyist for this mistake, since many of the pet shops from which the hobbyists buy their bettas display them on a shelf in cups or bowls left at room temperature? Bettas, in fact, do require warmer waters if they are to thrive. Aquaria should be heated to between 74° and 79°F for optimal comfort and metabolic efficiency. At temperatures below 70°F, bettas enter a state of minimal activity; their metabolic processes slow to a snail's pace, and the fish limits its physical movements to only what is absolutely necessary (picture the fish just lying on the bottom until it rises for a gulp of air).

Ironically, this metabolic stasis is largely responsible for the popularity of the betta bowl environment. If it moves very little and is slow to emit organic wastes, a betta is more easily housed in smaller quarters. And of course, since the typical betta bowl is too small to contain an aquarium heater, the water temperature rarely reaches the upper 70s, at which the betta's systems kicks into a higher gear, and more space/volume of water would be necessary. So limited space and lower temperatures are co-requisite to one another if the betta is to be "successfully" housed in a miniature aquarium or bowl. But is chilling the animal into inactivity what we want to do?

At temperatures above 81°F, the exact opposite scenario will occur. Moving about at rapid rates, and processing biological wastes quickly, the betta's body functions at an accelerated rate and causes the fish to age more quickly than it would when housed at cooler temperatures. Thus, the life expectancy of a betta housed in excessively warm waters is shorter than that of those individuals kept in cooler conditions. Again, the answer here is balance. Negligence of the temperature at which you betta is housed—either at the high end of the spectrum or the low end—is a practice that will cost your betta years off its life.

Proper Diet

A final point of contention among betta keepers is the matter of diet. Some hobbyists feed tropical flakes, while others go to great extremes to feed only the finest organic foods to their fish. In this instance, I must fall into the purists' camp. Unlike a great many tropical fish families (Cyprinidae, Gyrinochelidae, Loricariidae, etc.) who will take both vegetative and animal-based foods, the bettas are powerfully carnivorous, their native fare being fish fry, crustaceans, insect larvae, and minuscule worms. The optimum captive diet, therefore, should cater to the high protein needs of the betta. Avoid feeding regular fish flakes, spirulina, or other vegetative foods in favor of newly hatched brine shrimp, tubifex worms, white worms, mosquito larvae, daphina, or even very tiny bits of raw liver. Processed flakes, tablets, and pellets that are high in protein will also suffice.

Young bettas that are in a rapid stage of growth need particularly meaty fare, and lots of it too. Two small feedings per day is sufficient for growing bettas. Because the betta's bodily systems have adapted to process high quantities of animal protein, their growth rates, fin development, and vivid coloration all hinge upon receiving adequate prey items. Bettas that are fed vegetative matter may live their entire lives undernourished. The side effects of this malnourishment are low body weight and small size, unrealized finnage, and paler, less dynamic coloration.

A New Way of Thinking

The summation of all these efforts, the end product of our discussion here, is a general increase in the quality of life for your pet betta. By addressing and carefully controlling each aspect of captive husbandry (tank size, filtration methods, water current, temperature, and diet), you can optimize the life span, colors, health, finnage, and activity levels of your pet.

The old notions of a betta as a maintenance-free pet are simply incorrect. While a betta can survive in the unheated, unfiltered betta bowls of old, it cannot *thrive* under such conditions. The key to successful, long term betta health is to adjust your current community aquarium and modify it such that it will satisfy all your betta's captive needs, or to provide a small heated and filtered aquarium just for him. Then, and only then, will you ensure that your experiences with *Betta splendens* will be just as splendid as they can

APPENDIX D

Specimen	Temperature (C°) M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
B1	24.9	25.1	25.2	25.2	25.2	25.2	25.2	25.2	25.2	25.1
B2	25.2	25.1	25.2	25.3	25.2	25.4	25.3	25.2	25.4	25.3
B3	20.2	21.1	20.7	20.7	20.7	20.6	21.9	21.8	-	-
B4	20.1	20.9	20.5	20.7	20.6	20.4	21.8	21.7	21.4	21.3
B5	28.6	28.6	28.7	28.9	28.8	28.7	-	-	-	-
B6	28.8	28.6	28.8	28.9	28.9	28.7	27.5	26.4	26.1	25.6
B7	20.7	21	20.8	20.9	20.9	20.7	22.2	21.8	21.6	21.2
B8	20.6	20.9	20.8	21	20.9	20.7	22.2	21.8	21.5	21.2
B9	24.9	24.9	25.1	25.2	25.1	25.1	25.3	25	25.1	24.9
B10	20.9	21.4	21.3	21.4	21.4	21.1	22.6	22.1	21.8	21.5
B11	21	21.3	21.2	21.4	21.4	21.2	22.7	22.2	21.8	21.6
B12	20.7	21.1	21.1	21.4	21.2	21	22.2	22.1	21.7	21.6
B13	24.7	24.6	24.7	24.9	24.1	24.9	24.9	24.7	24.8	24.7
B14	24.9	21.6	24.9	25.1	24.9	25.2	25.1	24.7	25	24.7
B15	21.9	21.6	21.7	22.1	21.9	21.2	22.6	21.9	21.7	21.7
B16	28.2	27.7	28.1	28.4	28.1	28.1	27.6	25.5	-	-
B17	28.1	28	28.1	28.4	28.1	27.9	26.4	25	25.9	-
B18	28.2	28.1	28	28.3	28	-	-	-	-	-
B19	22.1	21.9	21.9	22.1	22.1	21.9	23	22.2	22.2	22.1
B20	22.2	21.1	22.1	22.2	22.1	22.1	23.1	22.3	22.3	22.1

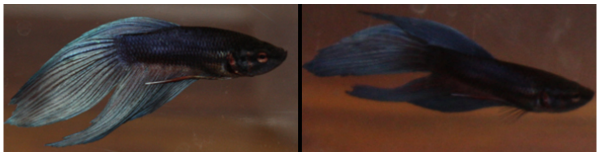
	pH									
Specimen	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
B1	6.17	6.2	6.2	6.16	6.12	6.22	6.33	6.16	6.08	6.14
B2	6.28	6.1	6.2	6.16	6.12	6.2	6.33	6.16	6.08	6.13
B3	6.29	6.2	6.3	6.19	6.17	6.25	6.35	6.26	-	-
B4	6.22	6.2	6.3	6.19	6.16	6.24	6.36	6.23	6.15	6.16
B5	6.04	6.1	6.2	6.1	6.07	6.12	-	-	-	-
B6	6.04	6.1	6.2	6.09	6.08	6.12	6.28	6.14	6.13	6.13
B7	6.12	6.1	6.3	6.17	6.15	6.22	6.33	6.23	6.16	6.15
B8	6.1	6.1	6.3	6.17	6.14	6.25	6.33	6.23	6.17	6.16
B9	6.09	6.2	6.2	6.14	6..11	6.1	6.31	6.24	6.18	6.14
B10	6.11	6.2	6.3	6.15	6.13	6.24	6.32	6.26	6.22	6.16
B11	6.06	6.2	6.2	6.16	6.13	6.23	6.32	6.25	6.24	6.18
B12	6.02	6.2	6.2	6.16	6.15	6.25	6.33	6.28	6.23	6.18
B13	5.98	6.2	6.2	6.1	6.13	6.19	6.31	6.27	6.23	6.18
B14	5.96	6.2	6.2	6.1	6.13	6.2	6.31	6.25	6.22	6.18
B15	5.97	6.2	6.2	6.09	6.14	6.21	6.31	6.24	6.24	6.22
B16	6.16	6.1	6.1	6.04	6.11	6.1	6.26	6.2	-	-
B17	6.14	6.1	6.1	6.04	6.12	6.11	6.27	6.23	6.25	-
B18	6.13	6.1	6.1	6.01	6.13	-	-	-	-	-
B19	6.15	6.2	6.2	6.06	6.22	6.19	6.32	6.27	6.27	6.23
B20	6.12	6.2	6.2	6.05	6.23	6.19	6.33	6.29	6.27	6.24

Specimen	M1	M2	M3	M4	NH_4^+ (ppm) M5	M6	M7	M8	M9	M10
B1	-	0.3	0.3	0.2	0.2	0.6	0.3	0.2	0.299	0.3
B2	-	0.2	0.2	0.2	0.2	0.5	0.3	0.1	0.1988	0.2
B3	-	0.2	0.3	0.2	0.2	0.7	0.8	0.1	-	-
B4	-	0.2	0.2	0.2	0.2	0.6	0.7	0.1	0.299	0.3
B5	-	0.2	0.2	0.1	0.1	0.8	-	-	-	-
B6	-	0.2	0.2	0.1	0.2	0.7	0.8	0.1	0.299	0.3
B7	-	0.4	0.3	0.3	0.2	0.6	0.9	0.1	0.39992	0.3
B8	-	0.4	0.3	0.3	0.3	0.6	0.8	0.1	0.3992	0.3
B9	-	0.4	0.2	0.1	0.1	0.5	0.8	0.1	0.1988	0.2
B10	-	0.3	0.2	0.1	0.2	0.7	0.9	0.1	0.0986	0.3
B11	-	0.3	0.2	0.1	0.2	0.6	0.9	0.1	0.09986	0.3
B12	-	0.3	0.2	0.1	0.2	0.5	0.9	0.1	0.0986	0.3
B13	-	0.3	0.2	0.1	0.2	0.5	0.9	0.1	0.0986	0.2
B14	-	0.2	0.2	0.1	0.2	0.5	0.8	0.1	0.0986	0.2
B15	-	0.3	0.3	0.2	0.4	0.8	1.4	0.2	0.0986	0.3
B16	-	0.3	0.1	0.1	0.2	0.5	0.7	0.2	-	-
B17	-	0.3	0.1	0.1	0.2	0.5	0.8	0.1	-0.0016	-
B18	-	0.3	0.1	0.3	0.3	-	-	-	-	-
B19	-	0.4	0.2	0.2	0.2	0.7	0.6	0.1	-0.0016	0.3
B20	-	0.4	0.2	0.2	0.2	0.5	0.5	0.1	-0.0016	0.2

Note: The date of each measurement was taken were denoted by M1 (5/31/2015), M2 (6/10/2015), M3 (6/13/2015), M4 (6/19/2015), M5 (6/21/2015), M6 (6/23/2015), M7 (6/28/2015), M8 (6/30/2015), M9 (7/21/2015), and M10 (7/26/2015).

APPENDIX E

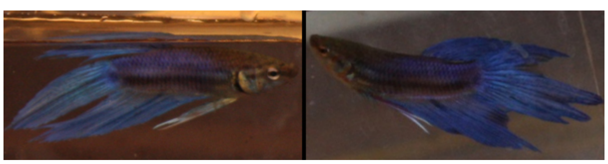
B1



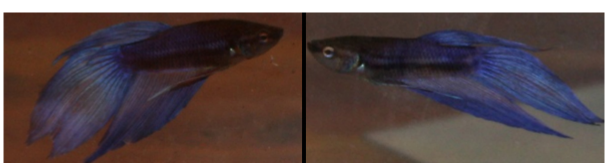
B2

B3

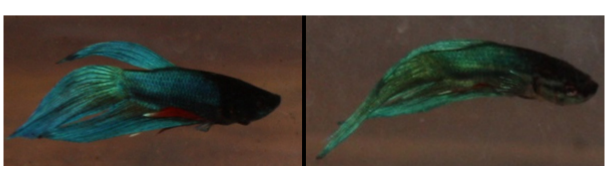
B4



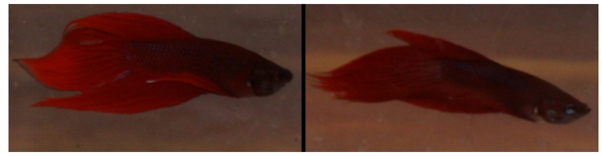
B5



B6

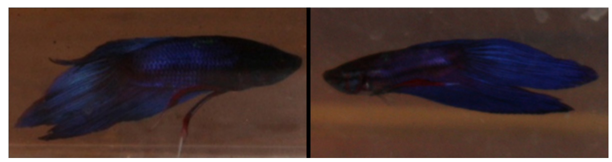


B7

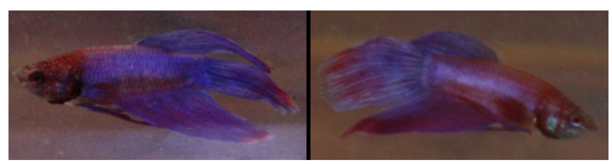


B8

B9

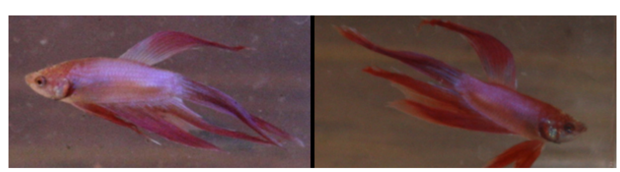


B10



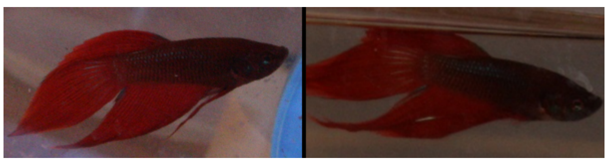
B11

B12

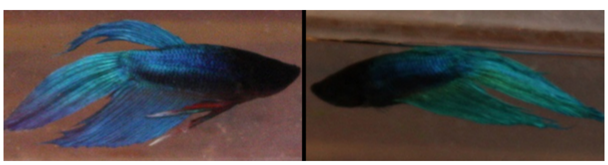


B13

B14

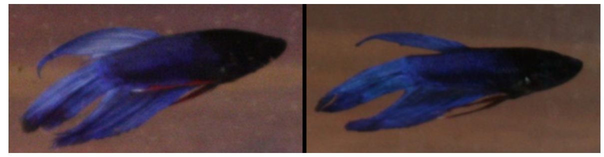


B15

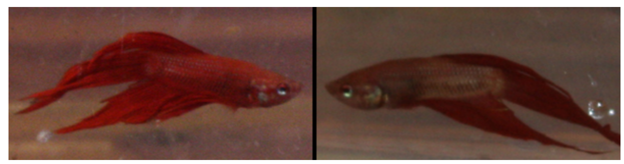


B16

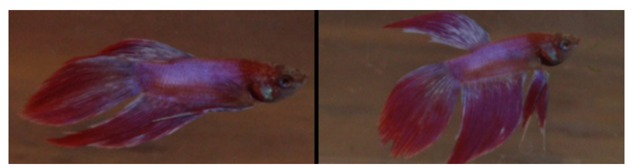
B17



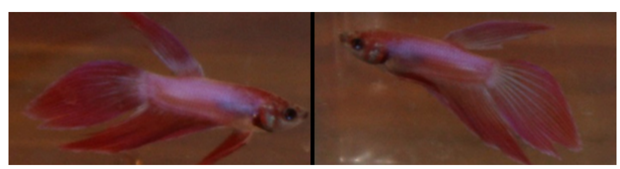
B18



B19



B20



APPENDIX F

MARYVILLE COLLEGE INSTITUTIONAL ANIMAL CARE & USE COMMITTEE
Application for Use of Vertebrate Animals in Student Research

Provide information after each bold item
Student Name:
Arielle Dolan
Student Email Address:
arielle.dolan@my.maryvillecollege.edu
Date:
1/14/15
Senior Study Advisor:
Dr. Drew Crain
Species to be used:
Betta splendens
Age of animals:
Unknown
Number of animals in study:
20
Duration of study:
2.5 months
Location of animals during the study (building and room):
304 Carson Creek Rd.
Limestone TN, 37681

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Arielle Dolan	423-737-4884		
Dr. Drew Crain	865-981-8238		

What will happen to the animals at the end of the study? If euthanasia is required, state the specific methods.
Animals will be rehomed to approved primary education classrooms and students of Maryville College.

(Do not write below line: For MC IACUC Use)

Maryville College IACUC Approval Number: 201507
Date Approved: May 1, 2015
Signed:

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