Veiltail Betta Fish Growth Experiment
Performed by Biologist Katie Kopinski
Spring/Summer of 2012

I. Purpose

To compare the growth in body length of Veiltail Betta fish (*Betta splendens*) in tap water to that of Watt-Ahh® an AquaNew Water (manufactured by AquaNew, LLC located in Sarasota, FL.).

II. Hypothesis

The Veiltail Betta fish in Watt-Ahh® water will have a greater growth in body length when compared to that of the fish in tap water.

III. Protocol

A protocol was used based on scientific literature review and interviews with both professional aquarium experts and marine biologists at MOTE Marine located in Sarasota, Florida

IV. Materials

2 Aqueon Basic Kit Aquariums (10 gallons) including Aqueon Water Conditioner
3 boxes of Aqueon replacement filter cartridges (6 cartridges, size medium)
2 Aqueon Submersible Aquarium Heaters (50 Watt)
2 jw Aquatic Fusion Smart-temp Aquarium Thermometer
1 Sentry AQ Mardel Freshwater Biozyme (8g)
2 Petco Plastic Plants (small, foreground)
2 Petco Plastic Plants (medium, midground)
1 Petco Aquarium Gravel Bag (9 kg)
3 Sentry AQ Mardel 5 in 1 test strips
3 Kordon Smart Start Instant Water Conditioning Kit
1 Tetra TetraMin fish flakes
1 BiOrb Cleaner Pump
4 Zebra Danios (*Danio rerio*)
4 Leopard Danios (*Danio frankei*)
2 female Veiltail Betta Fish (*Betta splendens*)
Sarasota City Tap water
Watt-Ahh® an AquaNew® water

V. Procedure

A. Tank Preparation (Figures 1 and 2)

1. Place 10 gallon tanks on a stand evenly, so that all sides are supported.

2. Wipe the inside with freshwater; do not use any chemical products to clean the tank. Check the tank for cracks or leaks.
3. Rinse gravel in a colander before carefully pouring gravel into the aquariums. Spread gravel evenly around the bottom with a depth of 1-3 inches.
4. Rinse plants and décor with warm water before placing into the aquarium; both tanks should have the same placement. Cover the base of the plants and décor with gravel to hold items in place.
5. Pour room-temperature tap water over hand and into tank to avoid moving gravel, plants, or décor; fill tank to bottom edge of top frame. Add water conditioner which also de-chlorinates the water.
6. Repeat step 5 using Watt-Ahh®.
7. Rinse the filter cartridges with cold water before assembling the filter according to included instructions. Place filter in desired location on back frame of tank.
8. Adjust heaters to appropriate temperature for the fish (78°F) and suction to back of each tank.
9. Attach thermometer next to heater.
10. Add Deluxe Full Hood to top of aquarium. Plug in filters, heaters, and hoods into outlet.
11. Add a pinch of Biozyme to each aquarium.
12. After acclimating 2 Zebra Danios and 2 Leopard Danios (for each tank), allow the nitrogen cycle to take its course for 4-6 weeks.
13. After the cycle is complete, acclimate and add one female Veiltail Betta Fish to each tank.

B. Acclimation

1. Clip Petco bags on side of tank in which the bags are in contact with the new aquarium water (Figure 3). For Betta Fish, let the plastic containers float in the water.
2. Let the bags sit for 20-30 minutes before placing the new fish into the tank.

C. Feeding

1. Feed fish in both tanks a pinch of TetraMin Flakes.
2. Feed each tank once at 8:00 a.m and once at 5:00 p.m.
3. Once a week, skip a day of feeding. This prevents the fish from having constipation.

D. Measuring Fish

1. Scoop Betta Fish into small container with tank water.
2. Using fish net, place Betta fish on paper and mark from mouth to base of body.
3. Place Betta Fish back into tank and measure the marked distance (in cm) with a ruler.

E. De-chlorination of Tap Water and Water Changes

1. To ensure de-chlorination, Aqueon Water Conditioner was added to each of the initial water types used to fill each of the 10-gallon tanks.

2. Once a week, turn off the filters and heaters in the tanks.
3. Clip the non-siphon end of the Cleaner Pump to a bucket (Figure 4).
4. Squeeze the pump enough times so that water will begin flowing through the hose.
5. Move the nozzle around in the gravel to suck up debris; stop after 1 gallon has been removed (slightly more can be removed if necessary) (Figure 5).
6. In clean buckets or jugs, insert the replacement water and amount (tap water for the tap water tank and Watt-Ahh® for the Watt-Ahh® Tank)
7. With the new water, add a pinch of Biozyme, 5 ml of Water conditioner and allow the water to sit for 15 minutes before adding to each of the tanks.
8. If necessary, add 1 tsp. AmQuel Plus to each tank to lower ammonia levels.
9. If necessary, add 14-20 drops Accu-Clear to each tank to remove cloudiness
10. Over the study period, water was added a least 6 times into each tank that ranged in volume between one and two gallons.

F. Water Parameter Measurements

1. Using Sentry AQ Mardel 5 in 1 test strips, place one test strip in each tank for 5 seconds.
2. Remove strip and allow 30 seconds to pass before comparing strip to bottle examples of pH, hardness, and alkalinity levels (Figures 6 and 7).
3. Allow 30 more seconds to pass before viewing the Nitrate and Nitrite levels (Figure 8).
4. Record results in a Microsoft Excel Spreadsheet.
5. Perform this task on a daily basis.

G. Changing Filters

1. Turn off filters and heaters.
2. Remove old filter and throw away.
3. Rinse Aqueon replacement filter cartridges in cold water to remove carbon dust.
4. Place new filter cartridge back in position

VI. Data Collection

A. Nitrogen Cycle

Two similar 10-gallon aquariums were prepared as described in Section V.A. above. After acclimating a total of four fish for each tank (two Zebra Danios and two Leopard Danios), the nitrogen cycle was allowed to take its course for 4 to 6 weeks. On a daily basis between April 11, 2012 and June 24, 2012, levels of nitrate, nitrite, water hardness, alkalinity and pH were measured using test strips and data results were recorded for each tank.

B. Fish Growth Experiment

Once the nitrogen cycle was completed, the fish growth experiment lasted for one month between May 27, 2012 and June 24, 2012. At the beginning of the experiment, one female Veiltail Betta joined the two Zebra and two Leopard Danios for each

aquarium tank. Female Bettas were chosen because they typically have shorter fins than male Bettas, limiting the possibility of the Danios nipping at Betta fins (thereby compromising the health of the fish and the results of the experiment). Since Betta fish are not compatible with other Bettas, Danios were chosen for each tank to accompany them. The environments for the two tanks were identical, the feeding and cleaning regiments the same. Both female Beta fish measured 2.8 cm in length at the beginning of the experiment. The only difference was the use of Watt-Ahh® in one tank and de-chlorinated tap water in the other. Each fish was measured for length approximately each week during the experiment period as described in Section V.D. or a total of five times (see Table 2).

VII. Results

A. Water Filters

When changing filters the first few weeks, the cartridges in Watt-Ahh® were slightly cleaner than the cartridges in the tap water (Figures 9 and 10).

B. Water Parameter Testing

Low nitrate levels reduce the risk for infection in fish. Using test strips, the water parameter results showed a noticeable difference between the two types of water used in the separate tanks. Throughout the fish growth experiment, nitrates within the tap water tank ranged between zero and 20 mg/L while the "Watt-Ahh® tank" was stabilized with zero readings on the test strips.

The nitrite levels in the tap water tank varied between 0 and 3 during the first two weeks of the fish growth experiment (between May 27, 2012 and June 10, 2012). A measurement of "3" for nitrites is identified on the test strip color spectrum as potentially causing "stress" for the fish. For the rest of the time during the fish growth experiment, no nitrites were detected using the test strips. For the Watt-Ahh® tank, no nitrites were detected throughout the entire experiment. The poor nitrite condition of the tap water during the initial weeks of the experiment may have contributed to the lower growth in length for the tap water fish.

For example, during the second week of the fish growth experiment (June 10, 2012), the parameters showed slightly higher nitrate levels and increased water hardness for the tap water tank, while the test strip results detected no nitrate, nitrite or hardness in the Watt-Ahh® water tank as shown in Table 1 and Figure 11. The Watt-Ahh® tank remained consistently stable throughout the fish growth experiment such that no nitrite detection was evident on the test strips. Total alkalinity tested zero for both types of water.

	Nitrate ppm (mg/L)	Nitrite ppm (mg/L)	Total Hardness (ppm)	Total Alkalinity (ppm)	pH
Tap Water	0-20	0	120	0	6.8
Watt-Ahh®	0	0	0	0	6.4

Table 1. Water Parameters for both tanks during second week of the fish growth experiment period (June 10, 2012).

C. pH

Both aquariums were exposed to air which is known to affect the pH. The only parameter that varied in the Watt-Ahh® tank was pH that was slightly more acidic when compared to that of tap water (between 6.4 and 6.8 as indicated on test strips).

D. Fish Growth Results

During the first week of the experiment, there was a growth spurt of 14.3% growth increase in length for the fish in the Watt-Ahh® tank and a 7.1% increase for the fish in the tap water. Another growth spurt for the Watt-Ahh® fish occurred during the last week of the experiment. At the end of the experiment, the Watt-Ahh® fish had a total of 28.6% body length increase while the tap water fish had a body length increase of 17.9% (Table 2 and Figure 12).

	Fish Length(cm)	
	Tap Water	*Watt-Ahh®*
5/27/2012	2.8	2.8
6/3/2012	3	3.2
6/10/2012	3.1	3.3
6/17/2012	3.2	3.3
6/25/2012	3.3	3.6

Table 2. Fish lengths for one month

VIII. Discussion

Shortly after acclimating the Zebra Danios and Leopard Danios, it was observed that the fish in the tap water tank remained at the bottom. However, the fish in the Watt-Ahh® were noticeably more active and were swimming at all depths of the tank.

When changing filters the first few weeks, the Watt-Ahh® cartridges were slightly cleaner than the tap water cartridges (Figures 9 and 10). The water parameters also showed a noticeable difference. The Watt-Ahh® water was slightly more acidic than anticipated for ultra pure water. This could be caused by the fact that there were other chemicals being added to the water, the water was exposed to the air, and measuring pH with a test strip is less accurate when compared to that of titration performed by a professional laboratory. For all other tested parameters, Watt-Ahh® remained stable and at zero throughout the experiment. The higher nitrite condition of the tap water may partly explain the significant growth spurt differential between the two fish during the initial week of the experiment (7.1% for the tap water fish vs. 14.3% for the Watt-Ahh® fish or double the body length increase for the latter fish).

At the end of the experiment, the Veiltail Betta Fish in the Watt-Ahh® tank showed a greater increase in total length (over 10%) than the fish in the tap water tank (3.6 vs. 3.3 cm for the latter fish). This result is significant when considering Veiltail Betta fish are typically a slow growing fish.

Additional studies involving more fish and different species will serve to quantify the additional biologic growth potential of Watt-Ahh®. It would be of interest to run a study such that the water-based gas used to produce Watt-Ahh® is directly bubbled into the tank itself and compare the fish growth with fish in a separate aquarium bubbled with air.

Tap Water Veiltail Betta Fish (*Betta splendens*), August 2012

Watt-Ahh® Veiltail Betta Fish (*Betta splendens*), August 2012

Figure 1. Experiment Set-up

Figure 2. Tank preparation

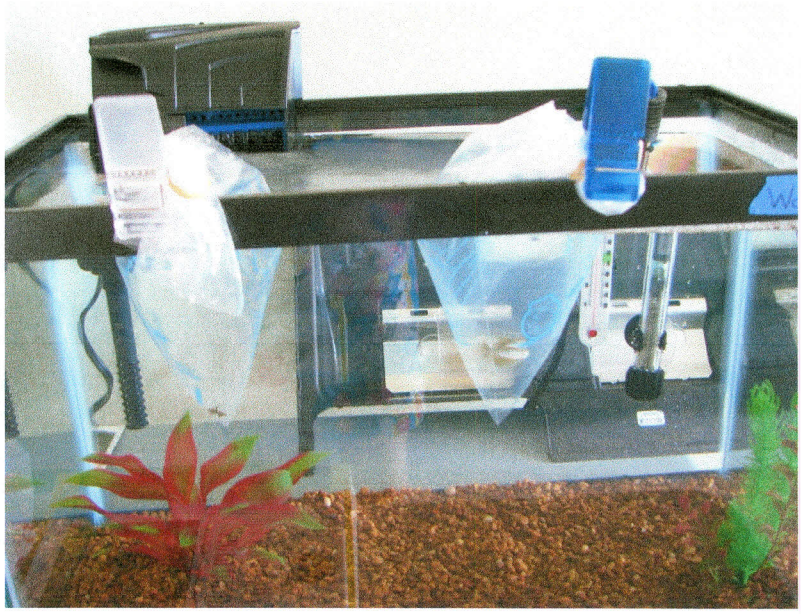

Figure 3. Acclimating the Zebra Danios and Leopard Danios

Figure 4. Attaching Gravel Vacuum hose to jug.

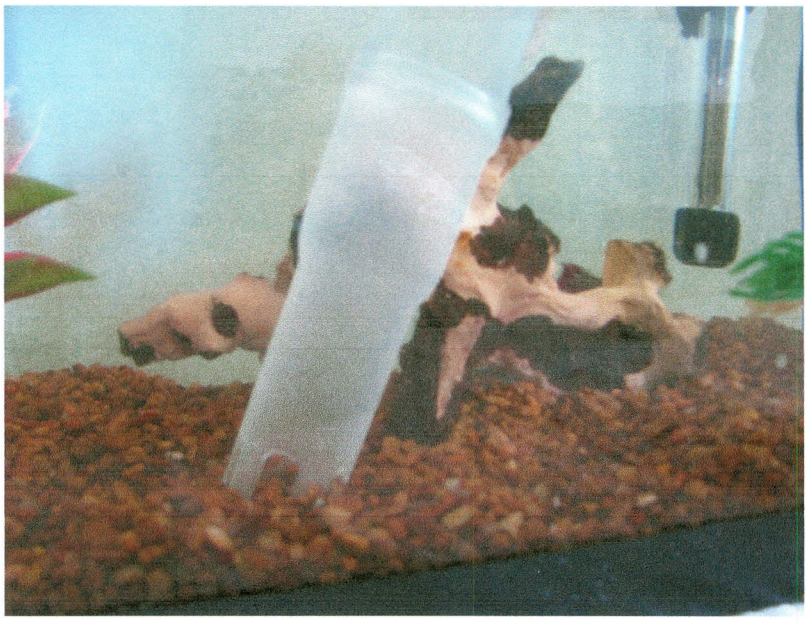

Figure 5. Cleaner pump siphon

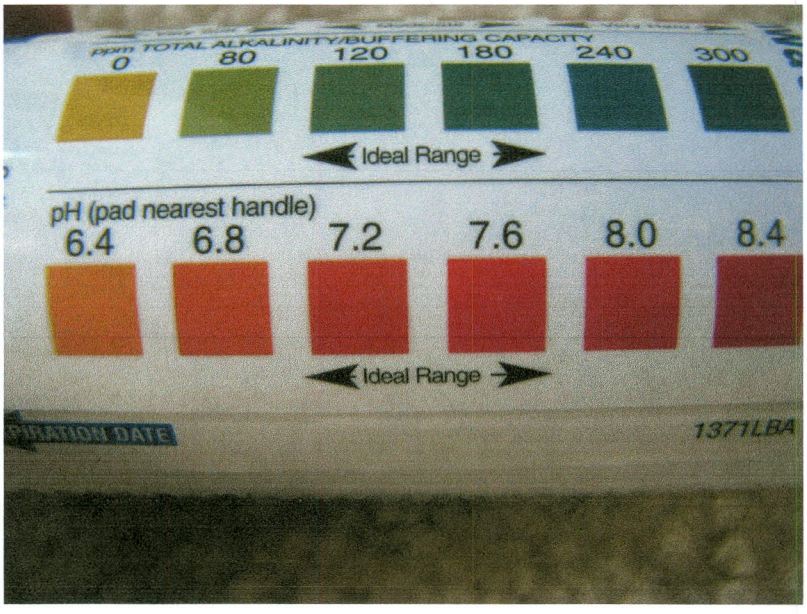

Figure 6. pH and Alkalinity ranges.

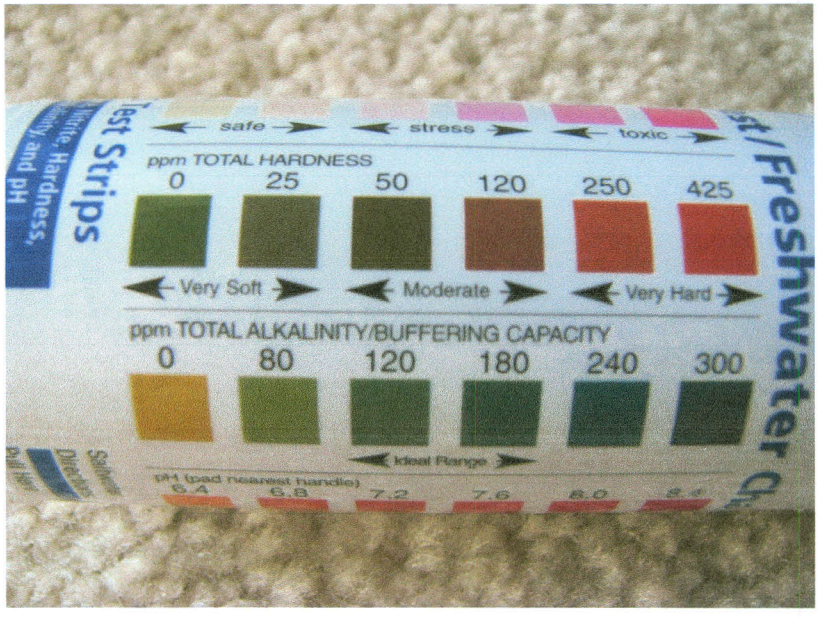

Figure 7. Alkalinity and Hardness ranges.

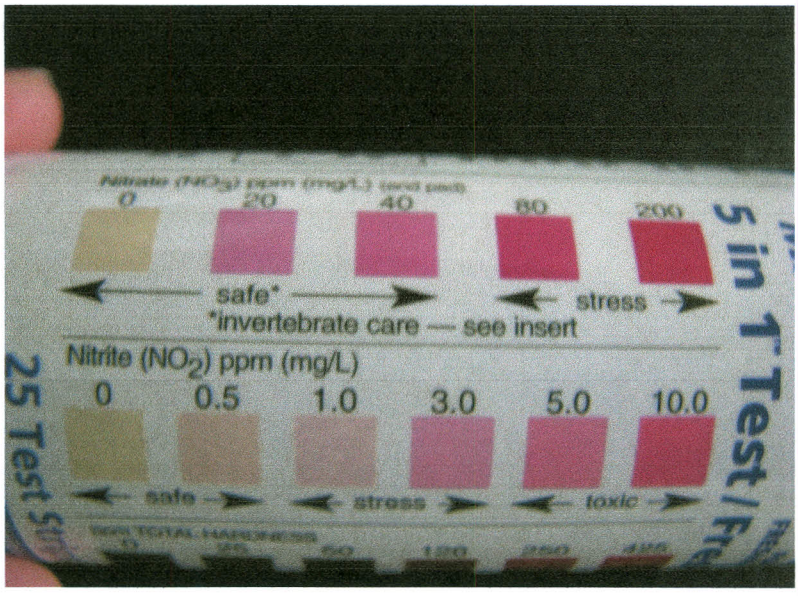

Figure 8. Nitrite and Nitrate ranges.

Figure 9. Watt-Ahh® filter cartridge. Week 1.

Figure 10. Tap Water filter cartridge. Week 1.

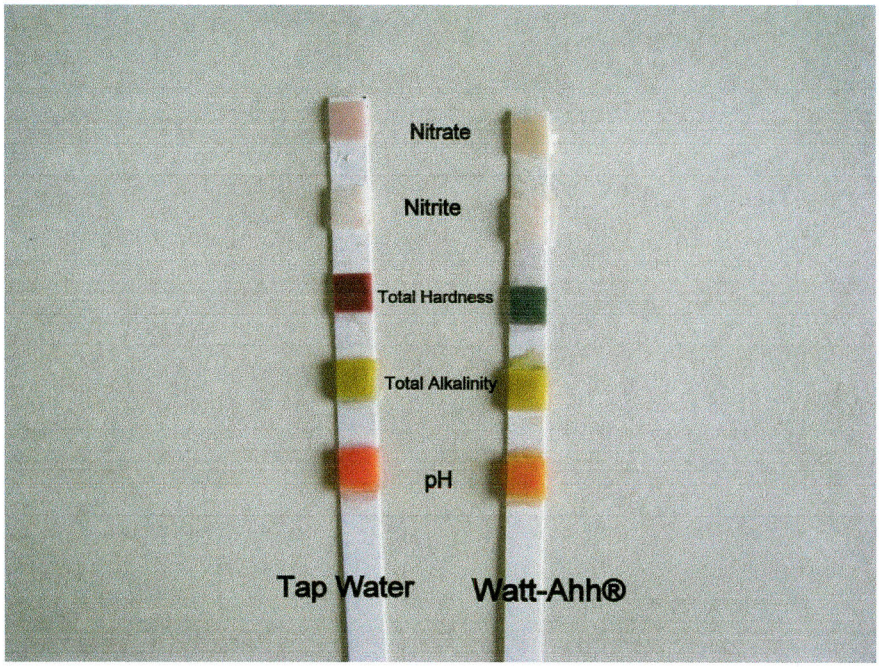

Figure 11. Water parameter comparison.

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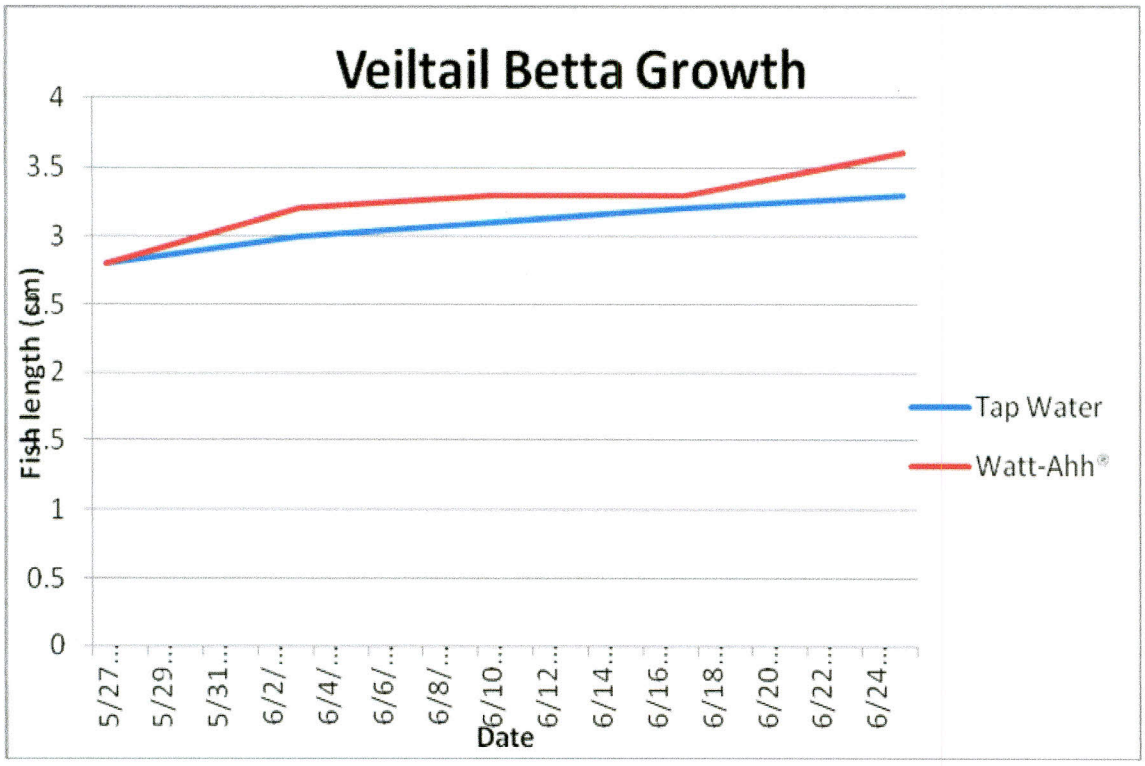

Figure 12. Veiltail Betta Fish growth in length

	Fish Length(cm)	
	Tap Water	*Watt-Ahh®*
5/27/2012	2.8	2.8
6/3/2012	3	3.2
6/10/2012	3.1	3.3
6/17/2012	3.2	3.3
6/25/2012	3.3	3.6

Table 2. Fish lengths for one month