

THE RELATIONSHIP BETWEEN COLOR PREFERENCE
AND NEST SITE SELECTION IN
THE SIAMESE FIGHTING FISH, BETTA SPLENDENS (REGAN)

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This is to certify that the
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James C. Bradlock

Major professor

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ABSTRACT

THE RELATIONSHIP BETWEEN COLOR PREFERENCE AND NEST SITE SELECTION IN THE SIAMESE FIGHTING FISH, BETTA SPLENDENS (REGAN)

by Richard H. Gude

Both color discrimination and nest site preferences have been demonstrated in Betta splendens. This study represents an attempt to determine if there is a relationship between nest site selection and color preference.

Fifty adult male Betta splendens, purchased locally, and maintained under laboratory conditions were used in these investigations. A total of 50 different test situations were studied. These involved the use of four colors of two intensities plus black, white and two shades of gray and were offered in pairs to the test individuals.

The testing situation consisted of ten aquaria, with two acetate discs floating equidistant from the sides and ends, with an attached stimulus aquarium centered on one side. Each aquarium was covered on the outside by opaque black plastic, except for an opening which allowed for visual contact between the fish in the test aquarium and the fish in the stimulus aquarium. Each was lighted continuously from above by a single 30 watt fluorescent bulb. All of the

test aquaria were placed in an enclosure, which excluded all daylight, and was maintained at a constant temperature. The nest site preference was determined by the number of individuals constructing bubble nests under each of the two colored discs presented during each 24-hour period. In one series all combinations of colors and intensities were tested. In a second series all high intensity colors and the black, white and two shades of gray discs were compared.

The data collected from these tests were subjected to various statistical analyses. These analyses indicated that yellow was preferred over green, red and blue, in that order. A preference was usually shown for the lower intensity color, but intensity did not govern preference. In the second series, colors were preferred over the black, white and two shades of gray. The order of preference for this series was yellow, green, red, light gray or dark gray, white, blue, and black.

Spectrographic analyses of pigment extracts of plant species normally used as nest sites in nature were made as well as spectrographic analyses of fish body pigments. These analyses were compared to those obtained from the plastics used in these investigations, and were found to have similar characteristics.

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INTRODUCTION

The ability of Betta splendens (Regan) to discriminate colors was first reported by Lissman (1932). Subsequently, Braddock and Braddock (1959) and Forselius (1957) have made extensive studies of the nest building behavior of B. splendens. Until now no attempt has been made to associate color with nest site selection. This study was made to determine whether or not such an association exists.

The ability to discriminate colors occurs among both invertebrates and vertebrates. Color discrimination has been studied more extensively among the vertebrates, however. Walls (1942), in his discussion of the vertebrate eye, stated that color vision is not present in all vertebrates and that it apparently has evolved separately in several vertebrate classes. Fishes are considered by most investigators to possess some type of color vision. Lissman (1932) conditioned B. splendens to respond to several colors.

The reproductive behavior of B. splendens has been the subject of much popular and scientific literature. Several investigators have made specific studies of the nest building behavior of the males. Braddock and Braddock (1959) described the type and manner of nest building. In later

papers, Braddock, Braddock and Kowalk (1960) found that, when offered floating discs of two sizes, the larger would be chosen as the site for the bubble nest. This occurred where the larger disc did not exceed 80 cm^2 . Braddock, Braddock and Richter (1961) found that male Siamese Fighting Fish could also discriminate certain shapes of floating forms and would show a preference for more compact shapes, such as discs, equilateral triangles, and squares. All of these were selected in preference to elongated rectangular forms. No evidence of discrimination among the compact shapes was observed.

This study represents an attempt to determine if any correlation exists between the color of a nest site and the preference for that particular nest site. Braddock and Braddock (1959), Forselius (1957), and Picciolo (1964) all agree that bubble nests serve as territorial markers in addition to their functions in connection with the rearing of young and the attraction of females. When it is a territorial marker, the bubble nest is located in the center of the territory. The presence of another male or a female causes increased nest building activity by a male that has started blowing nest bubbles. It is this natural behavior pattern that served as the basis for the work reported here.

Males to be tested were placed singly into a test aquarium and were in visual contact with another male. Two different colored discs were floated on the surface of the aquarium. Preference was measured in terms of choice of one color over another and also in terms of the ratio of square centimeters of nest bubbles deposited under each of the two discs. For a more thorough discussion of the reproductive behavior of B. splendens see Forselius (1957), and Braddock and Braddock (1959).

METHODS AND MATERIALS

Sixty adult male B. splendens were purchased locally and maintained in the laboratory for one month prior to testing. There was considerable variability among the individuals with regard to color. The color of each was noted and statistical tests were made to determine whether or not the color of the fish themselves correlated in any way with their preferences for disc colors. The size of all test individuals was very similar, ranging between 2.5 and 3.0 cm. standard length.

The fish were maintained in a special research laboratory located on the third floor of the Natural Science building at Michigan State University. Each individual was kept in a one gallon wide mouth bottle filled approximately $\frac{2}{3}$ full of water. This consisted of $\frac{2}{3}$ aged tap water and $\frac{1}{3}$ distilled water. The jars did not contain any gravel or vegetation. Each was cleaned periodically by straining out solid wastes and excess food. Water lost by evaporation was replaced with distilled water. Ten individuals were maintained in small one quart battery jars fitted with glass covers. These containers served as stimulus aquaria and will be described in detail below.

The mouths of the bottles were covered with individual sheets of .030 inch clear acetate plastic. The fish were isolated visually by opaque white cards placed between the bottles, which were stored on shelves. The fish were fed once a day small pieces of fresh ground shrimp. This daily diet was supplemented with brine shrimp nauplii.

The temperature of the laboratory was maintained between 80°-82° F. The laboratory was lighted by sixteen 40 watt fluorescent bulbs placed near the ceiling. All windows faced north and were covered by shades which effectively reduced daylight to a very low level. The overhead lights were maintained on a sixteen-hour on, eight-hour off cycle.

Ten test aquaria were placed on tables located inside a special enclosure 41 inches wide, 14 feet long, and five feet high. The inside of this enclosure was completely covered with flat black enamel. Ten 30 watt fluorescent bulbs were mounted perpendicularly to the long axis of the enclosure at 18 inch intervals. Located on the table, perpendicular to the long axis of the enclosure and centered directly beneath and parallel to each bulb was a 20 gallon test aquarium and its adjacent stimulus aquarium (Fig. 1). One side of the enclosure was removable. When closed the enclosure was light tight, that is, no outside light from

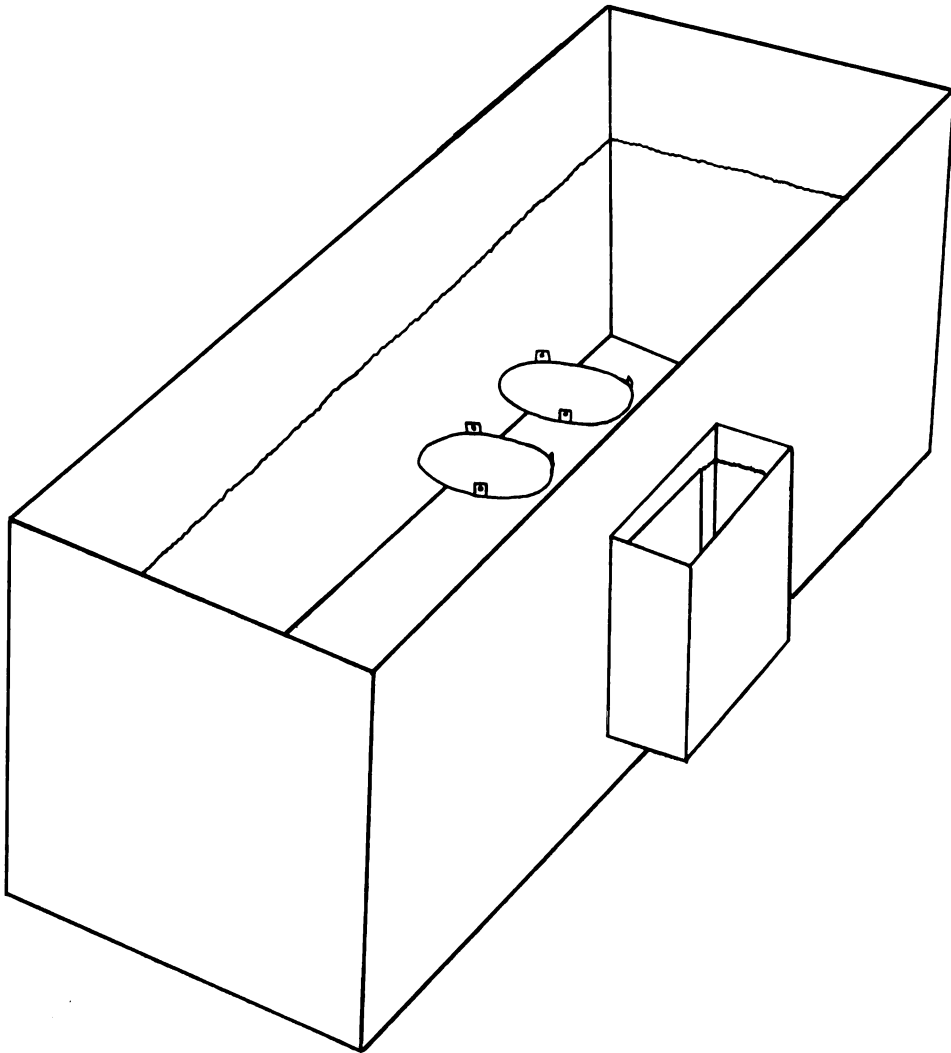


Figure 1. Test Aquarium and Stimulus Aquarium

the laboratory could reach the test situation. The test aquaria had a capacity of 20 gallons, and were 76 cm. long, 33 cm. wide, and 30.5 cm. deep. The bottom of each was covered with approximately one centimeter of black aquarium gravel. No vegetation was maintained in any of the test aquaria. Each was surrounded on all sides, externally, with opaque black plastic. The right side had an opening cut through this black plastic measuring 11.4 centimeters square and located five centimeters from the aquarium bottom and equidistant from both ends.

Each of the ten stimulus aquaria measured 15 cm. long, 15 cm. high, and 8.5 cm. wide. They were covered externally by translucent matte plastic on three sides. The fourth side of clear glass was placed against the opening on the right side of the test aquarium (Fig. 1). This allowed for visual contact between the individual in the stimulus aquarium and the test fish. The bottom of each stimulus aquarium was covered with one centimeter of black aquarium gravel, and was filled with water until the level corresponded to that of the test aquarium. The test aquaria were filled to a depth of 18 cm. with water ($\frac{2}{3}$ aged tap water, and $\frac{1}{3}$ distilled water).

The discs used as nest sites were cut from .010 inch

vinyl matte surfaced laminating plastic. The diameter of each disc was 11.3 cm. and the area was 100 cm^2 . Each disc had three tabs bent perpendicularly to the plane of the disc. Each tab had a hole near the end and all three were tied together with fine white thread which was looped over a fine wire hook suspended from the glass covering the test aquarium. The discs were floated on the surface of the aquarium 2.5 cm. from the center and 9.5 cm. from each of the sides. Colored discs were placed on top of the matte surface supporting disc, giving the desired color. White translucent discs were also used on top of the colored discs to adjust the intensity.

Four .010 inch transparent acetate colors were used: red, yellow, blue, and green. Two different intensities of each color were used in the experiments: a low intensity transmitting two foot candles, and a high intensity transmitting four foot candles. Appendix 1 lists the numbers and colors of discs used to establish the desired intensity. The first series of tests, comparing the high intensity and low intensity color groups, were conducted in five of the test aquaria. A second series of tests, comparing the high intensity colors, and black, white and two shades of gray were run in the remaining five aquaria. Appendix 1 lists the intensities and number of discs used to establish these intensities.

The only light reaching the floating discs came from the fluorescent tube located directly above the center of each aquarium. To insure against reflected light, all reflective surfaces in the enclosure were covered by flat black enamel. The outer surfaces of the aquaria were covered with opaque black plastic; and the bottoms were covered by black aquarium gravel. The discs, therefore, emitted only transmitted light. Spectrographic analyses indicating the emission spectra for the fluorescent lights and for daylight, as well as a comparison of the two will be found in Appendix 2.

The high and low intensity groups were established to control the variable of transmitted light intensity. The high intensity color group consisted of one each of the four colors, transmitting four foot candles as determined by the Wesson model 756 light meter. The high intensity group was established by the addition of opaque white acetate plastic discs of .010 inch and/or .020 inch thickness. The low intensity color group transmitted two foot candles and was established in the same manner. A third group not involving color was also established. This consisted of an opaque black disc, a white disc transmitting 38 foot candles, a light gray disc transmitting four foot candles, and a dark

gray disc transmitting two foot candles of light. Appendix 1 lists the numbers of the various discs used to establish all three groups. A spectrographic analysis was run on all the shades of the plastic to determine the percent transmission of light at the various wavelengths. Table 1 lists a composite of these results for the colored plastics.

Table 1. Composite of Spectrographic Analyses of Plastics*

Color	Limits	Peak Intensity
Red	600-760 mμ	690 mμ
Yellow	500-760 mμ	700 mμ
Green	490-580 mμ	530 mμ
Blue	380-560 mμ	480 mμ

*See Appendix 3 for individual spectrographic recordings.

mμ = millimicron

Table 2 lists the standard wavelengths for the various colors of the spectrum. Complete spectrographic analyses for the colored as well as the translucent white plastics will be found in Appendix 3.

Two series of tests were run. The first compared the high intensity with the low intensity color group and was divided into four blocks of tests. The second series compared the black, white, and two shades of gray with each other and with the high intensity colors. This series was divided into two blocks of tests. Appendix 4 lists test

Table 2. Standard Wavelengths of the Visual Spectrum
According to Bard (1956)

Color	Limits of Transmission
Red	723-647 m μ
Orange	647-585 m μ
Yellow	585-575 m μ
Green	575-492 m μ
Blue	492-455 m μ
Indigo	455-424 m μ
Violet	424-397 m μ

m μ = millimicron

sequences used for Series One and Series Two.

Standard randomization techniques were used to determine the order of testing for the blocks as well as the individual tests within each block. Two discs, differing in color were suspended in each test aquarium. During each test the same two colors were used in all five test aquaria. The position of each colored disc was determined randomly, as well as the test aquarium to be used for each fish.

Twenty-five fish were tested in each of the two series; five per day for five days. The same five fish were tested on the same day of each five day test period (i.e. fishes numbered 501-505 were tested the first day, fishes 506-510 on the second day etc.). Therefore, the use of the same fish on each test day gave each fish a four day rest period between tests.

Two series were run simultaneously throughout these observations. At the beginning of each test day the five fish to be tested for each series were netted individually from their bottles and placed into their test aquaria. Each was introduced on the north end of its aquarium. The aquarium was then covered with clear window glass, and the open side of the testing enclosure was covered. The fish were allowed to remain in the test situation in visual contact with their stimulus fish in their adjoining stimulus aquaria for 24 hours. At the end of this period the enclosure was opened; one section of the glass aquarium cover was removed; and the size of each bubble nest, in square centimeters, was measured and recorded. The nest size was estimated by determining the proportion of the 100 cm.² disc covered by bubbles. A mirror was used to observe the under sides of the discs.

Each test fish was removed from its test aquarium and placed in its individual bottle. All were then fed brine shrimp nauplii. The discs were then removed from the test aquaria, cleaned to remove any bubbles, and then placed back into the same test aquaria. The cleaning of the discs allowed sufficient time for the fish to feed on the nauplii. All fish were then given small pieces of fresh, finely ground shrimp until satiated. The next five fish to be tested were

then placed into the test aquaria. The second series of tests were conducted in the same manner.

These observations were all made between March 19, 1964, and August 12, 1964.

RESULTS

Twenty-five individuals were tested in both Series One and Series Two, making a total of 50 individuals tested. In Series One each individual was tested with 28 different color combinations of two discs. In Series Two each individual was tested with 22 different combinations of two discs.

Both series contained individuals that did not complete all tests. Thus, during the 140 day period when the Series One tests were being run, five individuals died. During the 110 day period when the Series Two tests were being run, five individuals also died. In neither Series One nor Series Two did any of these individuals die during their daily test period. Those individuals that died were replaced by individuals that had been purchased at the same time and had been maintained under the same conditions as the test individuals. The data collected from these were not treated separately.

In order to establish which colors were preferred, the individual test data were analyzed two ways. The first was an analysis using the "t" distribution utilizing the matched observations technique. This indicates whether the difference between the size of the nests constructed under both

discs are significantly different from zero. The second, and more rigorous statistical test, was a non-parametric sign test. This test indicates whether the number of individuals constructing nests under one color, regardless of the nest size, is significantly different from those constructing nests under the other color in any one test situation. Statistical test results giving a probability for chance occurrence of less than 5% were considered significant.

These two methods of analysis tended to agree with each other in most cases, that is, if significance was found in one test for a particular color, the other test also indicated significance for the same color. In no case in either Series One or Series Two did the significance tests contradict each other. If both tests did not show significance for the same color, then one or the other showed no significant difference between the colors being tested. In test Series One, with statistical analyses for 28 tests, there were only five analyses where both the "t" test and the "sign" test did not agree. In test Series Two, with statistical analyses for 22 tests, there were only six analyses where both significance tests did not agree. In the following discussion of the test results only the more rigorous sign test analysis results will be discussed.

Table 3 indicates the results of the first block of testing in test Series One. These test situations consisted of pairing the four high intensity colors, in all possible combinations, with the exception of testing the same color against itself. In Block One yellow was preferred over red or blue. No preference was shown when yellow was tested with green. Green was also preferred over blue or red. Red was preferred over blue. It is interesting to note that in Block One of test Series One, no bubbles were blown under the blue discs.

Table 3. Preferences Shown When the High Intensity Colors Were Compared in Test Series One, Block One

			YELLOW
		GREEN	NONE
	RED	GREEN	YELLOW
BLUE	RED	GREEN	YELLOW

Block Two of Test Series One consisted of testing the high intensity color with its low intensity counterpart. A total of four tests were run. No preference was shown between the high and the low intensity yellow. In the

remaining three tests of this block the low intensity of each color was preferred over its high intensity counterpart (table 4).

Table 4. Preferences Shown When the High and Low Intensities of the Same Colors Were Compared in Test Series One, Block Two

	Color			
	YELLOW	GREEN	RED	BLUE
Preference for high intensity	o	o	o	o
Preference for low intensity	o	x	x	x

x = preference

o = no preference

Table 5 lists the results obtained from the tests in Block Three of test Series One. This block consisted of 12 tests comparing all combinations of the high intensity and low intensity colors, excepting those previously tested in Block Two. High intensity yellow was preferred over low intensity red and low intensity blue; however, no preference was shown for high intensity yellow when compared to low intensity green.

No preference was shown when low intensity yellow was tested with high intensity green. Significance favoring the

low intensity yellow was found when it was tested with either the high intensity red or the high intensity blue.

No significance was shown when high intensity green was tested against either low intensity red or low intensity blue. However, significance was shown for the low intensity green when it was tested against either the high intensity red or the high intensity blue.

Table 5. Preferences Shown When the High and Low Intensities of the Different Colors Were Compared in Test Series One, Block Three

		High Intensity Colors			
		YELLOW	GREEN	RED	BLUE
Low Intensity Colors	YELLOW		NONE	LOW YELLOW	LOW YELLOW
	GREEN	NONE		LOW GREEN	LOW GREEN
	RED	HIGH YELLOW	NONE		LOW RED
	BLUE	HIGH YELLOW	NONE	NONE	

No preference was shown when the high intensity red was tested against the low intensity blue. A preference was shown for the low intensity red when it was tested with the high intensity blue.

Block Four of test Series One compared the low intensity colors in all possible combinations, excepting with themselves (table 6). The results from this block of tests were the same as those of Block One (table 3) with regard to colors preferred. The yellow was preferred over red or blue. No preference was shown when yellow was tested against green. Green was preferred over red. Red was preferred over the blue.

Table 6. Preferences Shown When the Low Intensity Colors Were Compared in Test Series One, Block Four

			NONE
		GREEN	YELLOW
	RED	GREEN	YELLOW

Table 7 is a summary of tables 3 through 6. Without considering intensity, yellow was preferred in eight tests, green in seven, red in four, and blue in one test. In the last case this test was one comparing high intensity blue with low intensity blue. No preference was shown in eight test combinations.

Table 7. Summary of Color Preferences Demonstrated in Test Series One (All Blocks)

HIGH YELLOW		LOW YELLOW		HIGH GREEN		LOW GREEN		HIGH RED		LOW RED		HIGH BLUE		LOW BLUE		NONE
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Test Series Two involved pairing all possible combinations of black, white and two shades of gray. As in test Series One, there were six tests, and shades were not compared against themselves. The black, white and two shades of gray were also compared in all 16 possible combinations with the high intensity colors identical to those used in test Series One.

The analyses of the data were carried out in the same manner as those for test Series One. Table 8 indicates the results of Block A of test Series Two. There were only two test combinations where a preference for one of the shades was shown, dark gray and light gray were both preferred when either of them was tested against black.

Table 8. Preferences Shown When the Black, White and Two Shades of Gray Were Compared in Test Series Two, Block A

WHITE	LIGHT GRAY		BLACK
	DARK GRAY		
	NONE	NONE	DARK GRAY
			LIGHT GRAY
			NONE

Table 9 indicates the results of Block B of test Series Two. A preference was shown for yellow when tested against black, white and two shades of gray. Green was preferred when tested against white and black. No preference was shown when green was tested against either the light or dark grays. Red was preferred when tested against black, but no preference was shown when tested against white, light gray or dark gray. Blue was preferred when tested against black. No preference was shown when blue was tested against white. When blue was tested against light gray or dark gray, preferences were shown for light gray or dark gray respectively.

Table 9. Preferences Shown When the Black, White and Two Shades of Gray and High Intensity Colors Were Compared in Test Series Two, Block B

	BLACK	DARK GRAY	LIGHT GRAY	WHITE
YELLOW	YELLOW	YELLOW	YELLOW	YELLOW
GREEN	GREEN	NONE	NONE	GREEN
RED	RED	NONE	NONE	NONE
BLUE	BLUE	DARK GRAY	LIGHT GRAY	NONE

Table 10 summarizes tables 8 and 9. It is interesting to note that yellow was preferred in all combinations and that no preferences for black were demonstrated in any test combination.

One-way analyses of variance were run to determine if there were any significant differences for the size of the bubble nests blown: 1. in each of the five tanks, 2. under

Table 10. Summary of Preferences Demonstrated in Test Series Two (All Blocks)

				BLACK
			DARK GRAY	DARK GRAY
		LIGHT GRAY	NONE	LIGHT GRAY
	WHITE	NONE	NONE	NONE
YELLOW	YELLOW	YELLOW	YELLOW	YELLOW
GREEN	GREEN	NONE	NONE	GREEN
RED	NONE	NONE	NONE	RED
BLUE	NONE	LIGHT GRAY	DARK GRAY	BLUE

the discs in the north or south position in each of the five test aquaria (position effect), and 3. by fish of differing body colors. The above three analyses were run on the total results obtained for each color.

Table 11 lists these results for test Series One. The means for all categories are shown if a significant heterogeneity was noted. The results indicate only four instances where significant heterogeneity was found. All these were found in the high intensity color series. A significant heterogeneity was noted between fish of different body colors in the case of the high intensity yellow, green or blue. In addition to this the sizes of the nests built in each test aquarium showed a significant heterogeneity in the case of high intensity green.

In test Series Two there was only one case where a significant heterogeneity was noted. This occurred in Block B when the data collected under the blue discs were analyzed. There was a significant difference in the size of the nests blown in the test tanks. Table 12 lists the means for each tank. These means were extremely low and the significance noted was not considered important.

In addition to the statistical analyses mentioned, these data were also compared as to the number of individuals

Table 11. Results of "f" Tests for Heterogeneity Comparing Size of Nest with Body Color of the Fish, Test Tanks and Positions of the Discs (Test Series One)

	COLORS	BODY COLOR OF FISH	SIZE OF NEST IN TEST TANK	POSITION OF DISC
High Intensity Colors	YELLOW	Green = 17.4 Red = 10.9 Blue = 7.6 Cream = 7.6	NONE	NONE
	GREEN	Green = 9.0 Red = 6.0 Blue = 2.8 Cream = 1.9	Tank 1 = 3.0 Tank 2 = 4.1 Tank 3 = 1.7 Tank 4 = 9.7 Tank 5 = 4.2	NONE
	RED	NONE	NONE	NONE
	BLUE	Green = .014 Cream = .0079 Blue = .0017 Red = .0000	NONE	NONE
Low Intensity Colors	YELLOW	NONE	NONE	NONE
	GREEN	NONE	NONE	NONE
	RED	NONE	NONE	NONE
	BLUE	NONE	NONE	NONE

Means for each category are listed if significant heterogeneity was noted.

NONE = no significant heterogeneity was noted.

Table 12. Results of "f" Test for Heterogeneity (Test Series Two)

	COLORS	BODY COLOR OF FISH	SIZE OF NEST IN TEST TANK	POSITION OF DISC
	BLACK	NONE	NONE	NONE
	DARK GRAY	NONE	NONE	NONE
	LIGHT GRAY	NONE	NONE	NONE
	WHITE	NONE	NONE	NONE
High Intensity Colors	YELLOW	NONE	NONE	NONE
	GREEN	NONE	NONE	NONE
	RED	NONE	NONE	NONE
	BLUE	NONE	Tank 1 = .13 Tank 2 = .58 Tank 3 = .75 Tank 4 = .73 Tank 5 = .3	NONE

Means for each category are listed if significant heterogeneity was noted.

NONE = no significant heterogeneity was noted.

responding to each color as well as the number of square centimeters of bubbles blown under each color.

In test Series One there were seven test situations for each color intensity. There were two intensities of each color, making a total of 14 test situations for each color. Each test situation consisted of results obtained from the testing of 25 fish, therefore, 350 fish contacts were made with each color.

In test Series Two there were seven test situations for the black, white and two shades of gray and four test situations for the high intensity colors. Each test situation consisted of results obtained from the testing of 25 fish. In all, 175 fish contacts were made with the black, white and two shades of gray, and 100 fish contacts with the high intensity color series.

Table 13 summarizes the responses made during these contacts for each color, intensity not considered. Listed are positive responses, those fish building the larger nests under the color being considered, negative responses, those fish building nests under the color not being considered, percentage of responses that were positive and the total number of responses.

In test Series One as in all other previous analyses,

Table 13. Total Number of Individuals Responding to the Various Discs Offered in Test Series One and Two

	COLOR	Total Number of Individuals Responding to Discs			
		Total Positive Responses	Total Negative Responses	% Positive Responses	Total Responses
Series 1	YELLOW	177	70	71.3	247
	GREEN	171	69	71.3	240
	RED	83	130	39.0	213
	BLUE	40	202	16.5	242
Series 2	DARK GRAY	63	46	57.8	109
	LIGHT GRAY	64	50	56.2	114
	WHITE	40	74	35.1	114
	BLACK	17	113	15.1	130
	YELLOW	75	7	91.5	82
	GREEN	54	21	72.0	75
	RED	46	19	70.8	65
	BLUE	24	53	31.2	77

the preferences for yellow and for green are approximately equal. Yellow and green are definitely preferred over red or blue, with red being preferred over blue. The color preferences shown in test Series Two are the same as those indicated in previous analyses. Light and dark gray are nearly equally preferred when the number of individuals responding to them is being considered.

The preferences shown for the four colors tested in Series Two, Block B, followed a slightly different pattern from previous analyses. A very strong preference was shown for yellow. The preference shown for green was substantially lower than the yellow. The preference for red was approximately equal to that for green. Blue was least preferred of all four colors.

Table 14 lists the total amount, in square centimeters, of bubbles blown under the various test discs. This table is set up in the same manner as table 13, except the percentage of positive response is based on the amount of bubbles blown under the color over the total bubbles blown both positively and negatively. This analysis agrees with the previous analyses as to color preferences and the preferences for the black, white, and two shades of gray.

Dark gray, which was the same intensity as the low

Table 14. Sizes of Bubble Nests Constructed Under the Various Discs Offered in Test Series One and Two

	COLOR	Total Square Centimeters of Bubbles Blown			
		Positive	Negative	% Positive	Total
Series 1	YELLOW	2603.5	1231.5	67.9	3835
	GREEN	2778	1247.5	69.1	4025.5
	RED	853	2069	29.1	2922
	BLUE	828.5	2514.5	24.8	3343
Series 2	DARK GRAY	1311	325	80.2	1636
	LIGHT GRAY	760.5	620.5	55.1	1381
	WHITE	324.5	825.5	28.2	1150
	BLACK	203.5	675	23.7	883.5
	YELLOW	865	173	83.5	1038
	GREEN	281.5	293.5	48.9	575
	RED	267	491.5	35.2	758.5
	BLUE	49.5	663.5	6.9	713

intensity color group was preferred over light gray, white, and black. Light gray, which was the same intensity as the high intensity color group, was preferred over both white and black. White and black were approximately equally preferred.

A one-way analysis of variance was run to determine if there was a significant difference between the various color intensities on the basis of amount of bubbles blown. When the values obtained for the high intensity colors of test Series One were totaled and tested against the totaled values for the low intensity colors of test Series One, a significant difference was noted. The sizes of the nests constructed under the high intensity colors were significantly smaller than those constructed under the low intensity colors, $F = 15.1$. When the high intensity colors of test Series One were compared by a one-way analysis of variance with the colors of test Series Two, a significant difference was also noted. The nests constructed under the high intensity colors used in test Series One were significantly larger than those constructed under the same high intensity colors used in test Series Two ($F = 29.9$). The high intensity colors used in test Series One were compared with the low intensity colors while the high intensity colors of test Series Two

were compared with the black, white, and two shades of gray. When the nest sizes obtained under the black, white, and two shades of gray were tested against those constructed under the high intensity colors used in test Series Two, no significant difference was noted ($F = .0085$).

When the nest sizes of all the bubble nests constructed in test Series One were compared with the nest sizes of all the bubble nests constructed in test Series Two, those of test Series One were found to be significantly larger than those of test Series Two ($F = 20.12$).

DISCUSSION

This investigation has demonstrated the ability of Betta splendens to discriminate certain colors and to show preferences for several of them. The latter appear to be valid color, and not merely intensity, preferences.

Definite color preferences were demonstrated in test Series One, which compared the high and low intensity color groups. Yellow and green were preferred over both red and blue. Intensity appeared to play a role in the preference, with the lower intensity of a given color generally being preferred over its higher intensity. Intensity did not, however, control color preference. Statistical analyses indicated that no preference was shown when the yellow discs were tested against the green, regardless of the intensity used. When the remaining tests in Series One were analyzed a subtle preference was indicated for the yellow over the green discs.

When the test combinations for both Series One and Two, not including any combinations of green against yellow, were considered, yellow was preferred in 12 of 13 combinations tested. When the test combinations for both Series One and Two, not including any combinations of green against

yellow, were considered the green was preferred in eight of thirteen combinations tested. The order of color preference shown was yellow, green, red, and blue.

When the black, white and two shades of gray were compared in Block A of test Series Two (table 8), very few preferences were noted. No preference was shown when the white and black were tested against each other. However, a preference was shown for the two shades of gray when tested against the black. This indicates that these shades of gray can be discriminated from black. The white-black test resulting in no preference indicates a strong negative stimulus value for the white. The fish must have been able to discriminate between them, as they were able to discriminate between either shade of gray and the black in other tests. No preference was shown for any of the shades except when they were compared against black.

The fish must also have been able to discriminate the white from the grays. When the two shades of gray and the white were tested against the black, preferences were shown for the grays and not the white. The fish demonstrated a strong avoidance of nest sites transmitting a very intense light or no light at all; such situations were considered to possess negative stimulus value. There is, however, a

region of intensity where the negative stimulus value is at a low level. This region apparently includes the two-four foot candles transmitted by the light and dark grays.

This apparent negative stimulus value demonstrated by the white and the black would have adaptive value in the natural situation. An opaque floating object would usually be relatively thick and necessarily dense. These properties would not lend themselves to the diffusion of respiratory gases for the developing young as a thinner and partially transparent leaf might. The white being very transparent might expose the parent as well as the nest to the attention of predators. This extreme transparent quality of the white would also allow large amounts of damaging solar radiation to reach the developing young, depending on the denseness of the leaf canopy overhead. It might also be misinterpreted by the fish as the water surface, devoid of a suitable nest site.

Forselius (1957) reported that in addition to serving as a point of attachment for the nests the vegetation also provides a shelter for the fry in the area around the nests and a means of protection for the adults against predators. Vegetation also serves as an important diurnal producer of oxygen, increasing the probability of the survival of the

eggs and fry. Vegetation also reduces rapid temperature fluctuations, and limits the penetration of harmful radiation. Mookerjee, Ganguly and Bhattacharya (1948) found that during periods of intense ultraviolet radiation, hatching time for the eggs of the Anabantid Ophiocephalus striatus was increased. Shading the eggs from ultraviolet radiation reduced hatching time.

When the black, white, and shades of gray were compared the order of preference shown was dark gray-light gray, white, and black. Testing the high intensity colors with the white, black and two shades of gray resulted in the following preferences: yellow, green, red-light gray-dark gray (equally preferred), white, blue, and black.

Several other investigators have studied the relationship between color preference and breeding site. Winn (1956) worked with three species of Darters (Percidae). His investigation indicated preferences were shown for texture and not the color of the egg laying site. Bilyi (1961) in his tests with the marine species of bream, sandre and sea roach also found no correlation between color and nest site chosen. In the case of the family Anabantidae the literature contains no reports dealing with the relationship between color preference and nest site selection. The ability of B.

splendens to discriminate between red and blue was established by Lissman (1932), but no correlation with nesting activity was reported.

Two hypotheses might be advanced to explain the color preferences shown. The first is a natural preference hypothesis, namely, that the fish show distinct preferences for the same colors transmitted by leaves under which they would build their nests in nature. The second is a negative preference hypothesis. The aggressive coloration of the fish is predominantly in the blue range with some red and a very slight amount of green. This second hypothesis would explain the results reported here as a negative preference for these "aggressive" colors, and therefore the nests were built under the "non-aggressive" colors, yellow and green.

According to the first hypothesis the fish were showing a preference for those colors normally used for nest sites in the native habitat. This species inhabits the waters associated with the alluvial plains of the Indochinese and Malay Peninsulas, where they are found in stagnant pools and streams, constructing their nests along the banks under floating aquatic plants (Tham, 1964).

Ladiges (1951) has found that many Anabantid species prefer areas of dense vegetation. Betta sp. and Trichogaster

sp. apparently prefer the more open water of the littoral zone bordering on areas of dense vegetation. Forselius (1957) has found that certain species of Anabantids, e.g. Trichogaster will incorporate fragments of the plants Azolla sp. and Salvinia sp. into their nests. These species vary markedly from B. splendens in this respect.

The general appearance of the plants used as nest sites by B. splendens varies considerably (Tham, 1964). For the purposes of this study they have been grouped into two artificial subdivisions based on leaf size. Eichornia sp. (water hyacinth) and Pistia sp. (water lettuce) are large leaved floating plants. The latter has leaves that are longer than broad, usually about ten centimeters long and five centimeters wide. The leaves of Eichornia sp. are rounder and slightly smaller. The plants found in the second group all have small leaves. The lobular shaped leaves vary from 0.5 centimeters to 1.5 centimeters in length. Azolla sp., Lemna sp., Salvinia sp. and Wolffia sp. are placed in this group. In addition to their small size these plants commonly form dense floating mats, with little or no space between adjacent leaves.

Certain of these genera are found in the United States and were obtained for spectrographic analysis. The remainder

were not available for analysis. Table 15 lists these analyses.

Table 15. Composite of Spectrographic Analyses of Plant Pigments

Plant	Limits of Transmission*	Wavelength of Peak Intensities*	
		First Peak	Second Peak
<u>Azolla</u> sp.	480-660 mμ	590 mμ	630 mμ
<u>Eichornia</u> sp.	490-650 mμ	520 mμ	630 mμ
<u>Lemna</u> sp.	470-700 mμ	570 mμ	650 mμ
<u>Pistia</u> sp.	480-660 mμ	520 mμ	630 mμ
<u>Salvinia</u> sp.	480-660 mμ	530 mμ	630 mμ

mμ = millimicrons

*See Appendix 5 for individual spectrographic recordings.

The range of the spectrum transmitted collectively by the yellow and green discs used in this investigation was between 490 and 760 mμ. A majority of the range of the spectrum transmitted collectively by the plants falls within these same limits (470-700 mμ). The wavelength of the peak intensity for the green discs (530 mμ) coincides approximately with the first peak intensity transmitted by Eichornia sp., Pistia sp., and Salvinia sp. The entire spectral range transmitted by the green discs, 490-580 mμ, coincides closely

with all of the first peak intensities transmitted by those plants analyzed. Perhaps even more significant is the total spectral range of transmission of the yellow discs, 500-760 m μ , which includes the wavelengths of both of the peak intensities displayed by all the plants analyzed. The data collected during this investigation suggest that the yellow is slightly preferred to the green. This is consistent with the hypothesis that the fish select nest sites that provide transmitted light most similar to that transmitted by plants that serve as nest sites in nature. The total spectral transmission for the blue discs, 380-560 m μ , also includes the wavelength of the first intensity peak for several of the plants. This overlap, however, occurs on a portion of the curve where the percent transmission of light at the plant peak intensity wavelengths is quite low, less than three percent.

According to the second hypothesis the fish were responding in a negative manner to particular wavelengths that are associated with certain aspects of their aggressive behavior, by not constructing their nests under discs transmitting these wavelengths. According to this hypothesis the fish would not demonstrate distinct preferences for particular wavelengths, but instead would build their nests

where there was a lack of color that possessed negative stimulus value. The colors most often displayed during periods of aggressive behavior are blue, blue-green and red. These terms remain rather nebulous unless spectral limits of transmission can be imposed upon them. An attempt was made to analyze light reflected from the tail fins of blue, blue-green and red fish. The light reflected from the red, however, was not adequate for analysis. The analyses of the remaining two appear in table 16.

Table 16. Composite of Spectrographic Analyses of Fish Skin Pigments

Fish Color	Limits of Transmission*	Wavelengths of Peak Intensities*	
		First Peak	Second Peak
Blue	360-540 mμ	430 mμ	480 mμ
Blue-green	380-560 mμ	450 mμ	480 mμ

mμ = millimicron

*See Appendix 6 for individual spectrographic recordings.

The spectral limits of transmission for the blue discs, 380-560 mμ, are synonymous with those of the blue-green individuals and include a majority of the spectrum of the blue individuals. The spectral limits of transmission of

the green discs was 490-580 m μ , which, while including a large portion of the spectral limits of transmission for both the blue and the blue-green fish, fails to include either of the wavelengths of peak intensities for either, although very close to the second peak. The limits of transmission for the blue discs include both of the peak intensities for both colors of individuals. In addition, the peak intensity for the blue discs coincides with the second peak intensity of both colors of individuals. These data are consistent with the hypothesis that the fish will respond negatively to a prospective nest site that transmits light corresponding to the aggressive body coloration, in this case, the blue discs.

An alternative hypothesis, equally valid on the basis of these data is, that the fish would respond in a negative manner to the blue discs, because the limits of transmission of the discs contain the peak intensities of the light emitted from blue sky (Appendix 2). The presence of such a transmission spectrum would indicate the complete absence of a suitable nest site.

Forselius (1957) reported that male B. splendens will display nuptial coloration in hostile situations. This threatening coloration tends to keep other males out of the

territory, keeping the area free from intruders during spawning and fry-rearing periods. The male will threaten any intruder. This threat towards a female will elicit mating behavior, thus insuring an increased number of contacts between males and females. Nuptial coloration, when displayed in the presence of another male, will elicit aggressive behavior from both individuals. This relationship between nuptial coloration and aggressive behavior also tends to support the negative preference hypothesis. There was, however, a portion of the spectrum that was reflected from the fish as well as transmitted by the green discs, which is not consistent with either hypothesis. This area of overlap may have imparted a slight negative stimulus value to the green which could possibly be responsible for the preference of yellow over the green. According to the second, a negative preference hypothesis, both yellow and green, or any other color lacking negative stimulus value would be equally preferred.

Tham (1964) stated that B. splendens occurs in areas where certain plants form dense mats of vegetation. These species are relatively few in number and the fish seem to associate with no others. This tends to support the natural preference hypothesis. However, there was a portion of the

spectrum transmitted by both the plant pigment extracts and the blue discs. This again is not consistent with either hypothesis, in that the fish, if responding positively to the plants as nest sites, would also be responding positively to a portion of the blue spectrum. However, this overlap occurs, as previously mentioned, in an area where the percent transmission of available light is very low.

There does not appear to be a specific stimulus controlling the color of the nest site selected by B. splendens, but rather a combination of at least two factors, a preference for nest sites approximating the spectral transmission of native sites (plants) and an avoidance of those nest sites transmitting light in the same spectral range as the body color which is associated with aggressive behavior, or sky color or factors unknown.

SUMMARY

1. Betta splendens were able to discriminate colors and demonstrated preferences for certain of them for nest sites.
2. Lower intensity colors were generally preferred over higher intensity colors, but intensity did not govern preference.
3. The order of colors preferred was yellow, green, red, and blue.
4. White and black possessed strong negative stimulus values.
5. When the colors were compared with the black, white and two shades of gray the order of preference was: yellow, green, red, dark gray-light gray, white, blue, and black.
6. It was concluded that Betta splendens choose the color of their nest sites due to the transmission of light normally transmitted by native nest sites, and, possibly a lack of light transmitted resembling aggressive body coloration, or the presence of light resembling the blue sky indicating the absence of a suitable nest site.

BIBLIOGRAPHY

- Bard, Philip (1956), Medical Physiology, 10 ed., 1421 p.
Mosby, New York, New York.
- Bilyi, M. D. (1961), Rol'kol'oru dna i substatu pry
rozshukuvanni plidnykamy ryb mists', prydatnykh dlya
nerestu, Dopovidi, Akod. Nauk. Ukrain, R.S.R. 10:
1389-1392.
- Braddock, James C., and Zora I. Braddock (1959), Nesting
behavior in the Siamese Fighting Fish, Brit. Jour.
Anim. Behav. 7:222-232.
- Braddock, James C., Z. I. Braddock, and G. Kowalk (1960),
Size discrimination in the Siamese Fighting Fish,
Betta splendens, Bull. Ecol. Soc. 41:82.
- Braddock, James C., Zora I. Braddock, and H. Richter (1961),
Form discrimination in Betta splendens, Am. Zool. 1(3):
345.
- Forselius, Sten (1957), Studies on Anabantid fishes, I, II,
and III, Zoologiska Bidrag Fran Uppsala 32:93-597.
- Ladiges, W. (1951), Der Fisch in der Landschaft., 2, Auflage,
182 p., Braunschweig.
- Lissman, Hans-werner (1932), Die umwelt des Kampffishes
(Betta splendens), Zeitschr. Vergleich. Physiol. 18(1):
65-111.
- Mookerjee, H. K., D. N. Ganguly and R. N. Bhattacharya
(1948), On the bionomics, breeding habits and develop-
ment of Ophiocephalus striatus, Block Proc. Zool. Soc.
Bengal 1:58-64. Calcutta.
- Picciolo, Anthony R. (1964), Sexual and nest discrimination
in Anabantid fishes, Ecol. Mono. 34:33-76.
- Regan, C. T. (1909), The Asiatic fishes of the family
Anabantidae, Prac. Zool. Soc. Land. 767-787.

Tham, A. K. (1964), Director, Fisheries Biology Unit,
Department of Zoology, University of Singapore. Personal communication.

Walls, G. L. (1942), The vertebrate eye and its adaptive radiation, Bull. Cranbrook Inst. Sci., No. 19, XIV + 785 p.

Winn, H. E. (1956), Egg site selection by three species of darters, Brit. Jour. Anim. Behav. 5(1):25-28.

APPENDIX 1

The following figures list the numbers of colored as well as white discs used to obtain equal intensity for all colors in both test series.

Number of Discs Used To Produce High Intensity Series*

Color	Number of Colored Discs	Number of .010" White Discs	Number of .020" White Discs
Blue	2	0	0
Green	2	0	0
Red	1	0	0
Yellow	3	1	2

*In addition to the supporting matte surface disc.

Number of Discs Used To Produce Low Intensity Series*

Color	Number of Colored Discs	Number of .010" White Discs	Number of .020" White Discs
Blue	2	1	0
Green	2	1	0
Red	1	1	0
Yellow	3	1	4

*In addition to the supporting matte surface disc.

Number of Discs Used To Produce the Black and White Series*

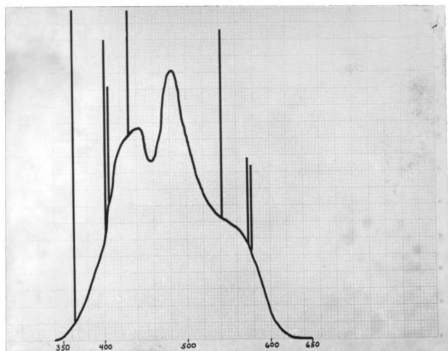
Color	Intensity (Foot Candles)	Number of .010" White Discs	Number of .020" White Discs
Dark gray	2	1	4
Light gray	4	1	3
White	38	0	0
Black	0	One .010 opaque black	-

*In addition to the supporting matte surface disc.

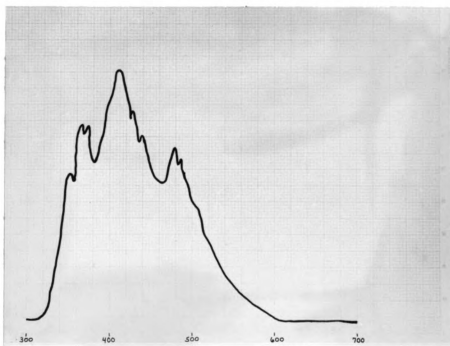
APPENDIX 2

The following are photographs of linear spectrographic emission records from the fluorescent tubes used in these experiments as well as north light which represents light normally reflected from the northern sky. These recordings were made by the investigator on a Bausch and Lomb Spectronic 505 recording spectrophotometer, owned by the Biology Department of Hartwick College, Oneonta, New York.

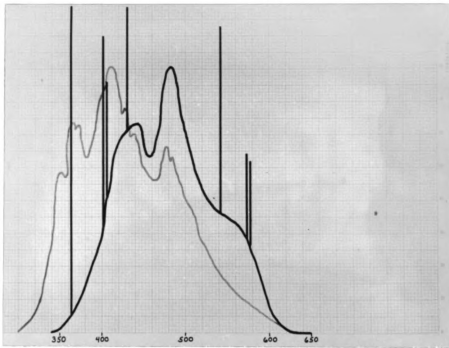
The ordinate represents the percent of light emitted. The abscissa represents the wavelengths in millimicrons of the light emitted. Full scale of the ordinate does not indicate 100 percent emission and no correlations can be drawn between these two recordings as to intensity of light emitted. The relative intensity of emission may only be compared at the various wavelengths on the same recording.



Spectral emission of fluorescent light



Spectral emission of day light (north light)

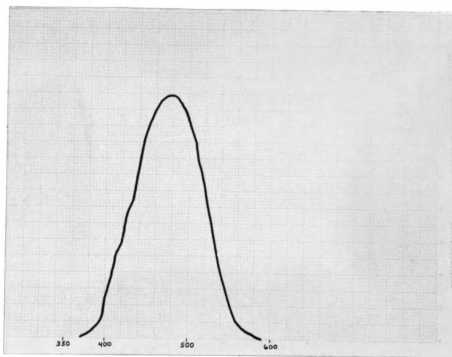


Comparison of spectral emission of daylight (light line)
and spectral emission of fluorescent light (dark line)

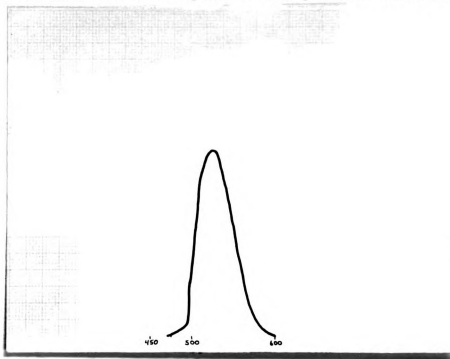
APPENDIX 3

The following are photographs of linear spectrographic transmission records of the various colored and white plastics used in these experiments. These recordings were made by the investigator on a Bausch and Lomb Spectronic 505 recording spectrophotometer owned by the Biology Department at Hartwick College, Oneonta, New York.

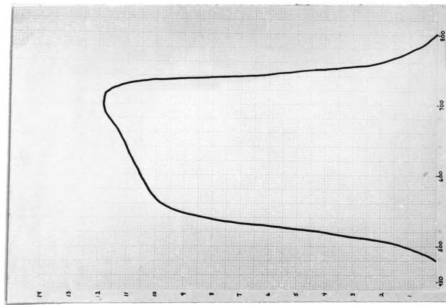
The ordinates represent the percent of available light transmitted. The abscissae represent the wavelength in millimicrons of the light transmitted. Unless indicated, the full scale of the ordinate represents 100 percent of available light transmitted.



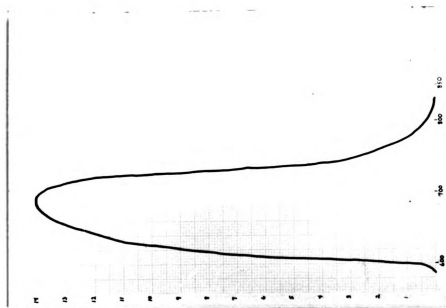
Spectral transmission of light through two sheets of blue and one of matte surface .010 inch acetate plastic. Full scale on the ordinate equal to ten percent transmission.



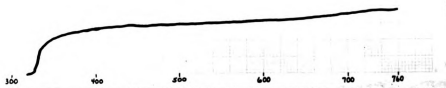
Spectral transmission of light through two sheets of green and one of matte surface .010 inch acetate plastic. Full scale on the ordinate equals ten percent transmission.



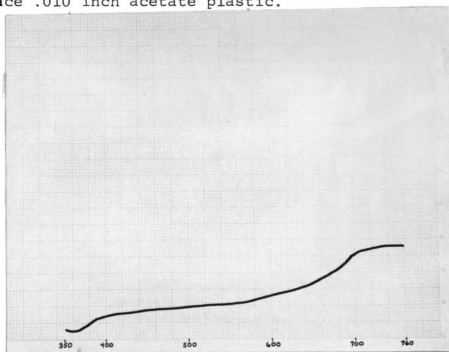
Spectral transmission of light through three sheets of yellow and one of matte surface .010 inch acetate plastic. Full scale on the ordinate equals fifteen percent transmission.



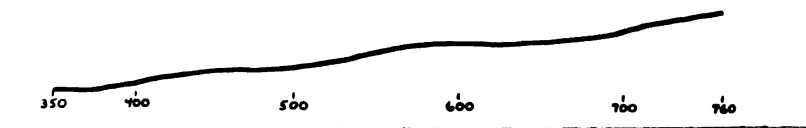
Spectral transmission of light through one sheet of red and one of matte surface .010 inch acetate plastic. Full scale on the ordinate equals fifteen percent transmission.



Spectral transmission of light through one sheet of matte surface .010 inch acetate plastic.



Spectral transmission of light through one sheet of white .010 inch acetate plastic. Full scale of ordinate equals ten percent transmission.



Spectral transmission of light through one sheet of white .020 inch acetate plastic. Full scale of ordinate equals ten percent transmission.

APPENDIX 4

The following tables list the sequence of testing. Both the order of testing for the blocks as well as the order of individual tests within each block were determined randomly. The blocks are listed according to order of testing. The number in the upper right hand corner of each cell indicates the order of testing within that block.

Test Series One, Block One

	Blue High	Red High	Yellow High	
Green High	6 Green High Blue High	1 Green High Red High	3 Green High Yellow High	
Yellow High	2 Yellow High Blue High	4 Yellow High Red High		
Red High	5 Red High Blue High			

Test Series One, Block Two

	Blue High	Red High	Yellow High	Green High
Blue Low	2 Blue Low Blue High			
Red Low		3 Red Low Red High		
Yellow Low			4 Yellow Low Yellow High	
Green Low				1 Green Low Green High

Test Series One, Block Three

	Blue Low	Red Low	Yellow Low	Green Low
Blue High		9 Blue High Red Low	2 Blue High Yellow Low	5 Blue High Green Low
Red High	11 Red High Blue Low		10 Red High Yellow Low	3 Red High Green Low
Yellow High	7 Yellow High Blue Low	1 Yellow High Red Low		8 Yellow High Green Low
Green High	6 Green High Blue Low	4 Green High Red Low	12 Green High Yellow Low	

Test Series One, Block Four

	Red Low	Yellow Low	Green Low
Blue Low	5 Blue Low Red Low	4 Blue Low Yellow Low	1 Blue Low Green Low
Red Low		2 Red Low Yellow Low	3 Red Low Green Low
Yellow Low			6 Yellow Low Green Low

Test Series Two, Block A

	White	Light Gray	Dark Gray	
Black	Black White 3	Black Light Gray 6	Black Dark Gray 2	
Dark Gray	Dark Gray White 5	Dark Gray Light Gray 4		
Light Gray	Light Gray White 1			

Test Series Two, Block B

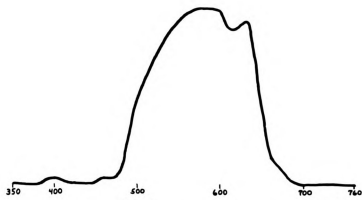
	Red	Blue	Yellow	Green
Black	Black Red 9	Black Blue 10	Black Yellow 16	Black Green 11
Dark Gray	Dark Gray Red 1	Dark Gray Blue 2	Dark Gray Yellow 15	Dark Gray Green 8
Light Gray	Light Gray Red 5	Light Gray Blue 13	Light Gray Yellow 14	Light Gray Green 3
White	White Red 12	White Blue 7	White Yellow 4	White Green 6

APPENDIX 5

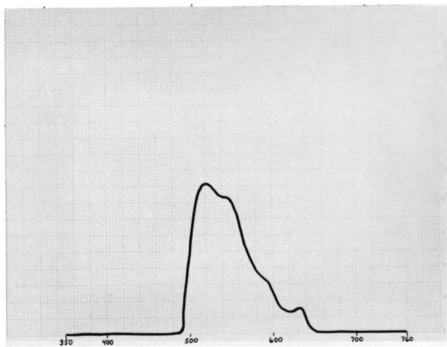
The following are photographs of linear spectrographic transmission records of the various plant pigment extracts discussed in this paper. These recordings were made by the investigator on a Bausch and Lomb Spectronic 505 recording spectrophotometer owned by the Biology Department of Hartwick College, Oneonta, New York.

The ordinates represent the percent transmission of available light. The abscissae represent the wavelengths in millimicrons of light transmitted. Unless indicated, the full scale of the ordinate represents 100 percent of available light transmitted.

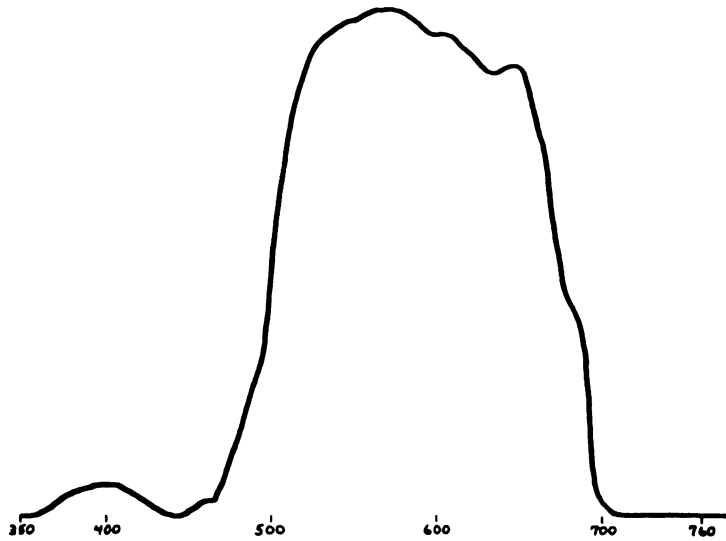
The extracts of leaf pigments were obtained by placing 25 grams of leaves and 75 milliliters of 95 percent ethanol in a Waring blender for one minute. This material was filtered. The filtrate containing the extracted pigments was compared to a blank containing 70 percent ethanol.



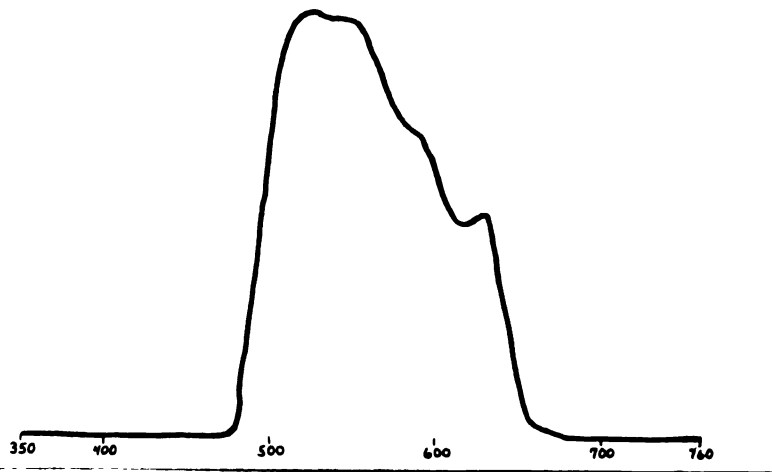
Spectral transmission of light through an alcohol extract of leaf pigments of *Azolla* sp.



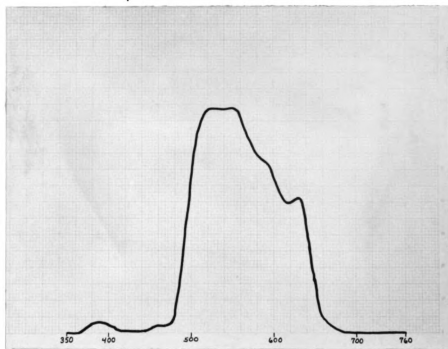
Spectral transmission of light through an alcohol extract of leaf pigments of *Eichornia* sp.



Spectral transmission of light through an alcohol extract of leaf pigments of Lemna sp.



Spectral transmission of light through an alcohol extract of leaf pigments of Pistia sp.



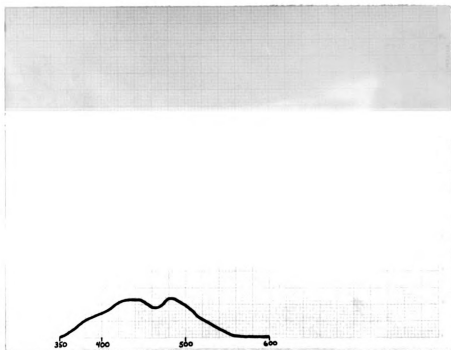
Spectral transmission of light through an alcohol extract of leaf pigments of Salvinia sp.

APPENDIX 6

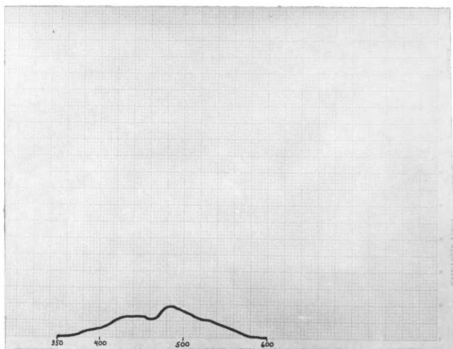
The following are photographs of linear spectrographic emission records from the tail fins of the blue and blue-green fish. These recordings were made by the investigator on a Bausch and Lomb Spectronic 505 recording spectrophotometer owned by the Biology Department of Hartwick College, Oneonta, New York.

The ordinates represent the percent of light emitted. The abscissae represent the wavelengths in millimicrons of light emitted. Full scale of the ordinate does not indicate 100 percent emission but is arbitrary. The only comparisons as to intensity that can be drawn must be made between these two emission spectra.

In order to obtain these data, the tail fins of fish were placed near the spectrophotometer on a black background. A light directed upon the fin reflected into the spectrophotometer for analysis. The recordings occurring below represent the total emission spectra minus the emission spectrum of the light source.



Spectral emission of light from the tail fin of a blue Betta splendens male.



Spectral emission of light from the tail fin of a blue-green Betta splendens male.

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