SOME OBSERVATIONS ON THE RELATIVE STIMULUS VALUES OF SIZE, SHAPE AND COLOR WITH RESPECT TO NEST BUILDING IN THE SIAMESE FIGHTING FISH, *BETTA SPLENDENS* (REGAN)

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THESIS

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ABSTRACT

SOME OBSERVATIONS ON THE RELATIVE STIMULUS VALUES OF SIZE, SHAPE AND COLOR WITH RESPECT TO NEST BUILDING IN THE SIAMESE FIGHTING FISH, *BETTA SPLENDENS* (REGAN)

by Michael O. Childs

It has been demonstrated that nesting male *Betta splendens* discriminates between discs of various sizes and shows preference for the larger disc. It is also known that discs are preferred to squares or triangles and that the colors yellow and green are preferred to red and blue and red is preferred to blue.

This study is an attempt to determine whether size, shape, and color have different values as stimuli in the nesting situation and if so, the relative value of each.

In testing the fish, a plastic disc having a preferred size but of a non-preferred color, was matched with another plastic disc having the preferred color but of a non-preferred size. Similar combinations were also made involving color and shape. Thirty-one fish used in groups of 20 were presented with 48 different combinations of size and color, and color and shape. It was then determined whether the stimulus of size overrode the stimulus of color or vice-versa and also whether the stimulus value of shape was

greater than the stimulus value of color. Shape and size combinations were not directly compared because it was hoped that their relative stimulus values would become apparent from the results of the tests actually performed.

The test apparatus consisted of ten, 20 gallon aquaria contained in a light-tight enclosure. These were covered with a layer of opaque black plastic with a small window on one side which allowed visual contact between the test fish and a fish maintained in a small stimulus aquarium adjacent to each test aquarium. A 30-watt fluorescent bulb was located directly above each test aquarium and was lighted continuously. The plastic forms serving as nest sites were floated in the center of the test aquaria. The preference shown by the fish was determined by the amount of bubbles deposited under a particular floating form during a 24 hour period.

The collected data were subjected to statistical analyses using the method of matched observations to test whether the mean of the differences was significantly different from zero. It was found that the fish generally showed a color preference with yellow most preferred, followed by green, red and blue regardless of the shape or size of the matching form.

Spectrographic analysis of pigment extracts from the fish and from plants normally used as nesting sites by the fish were compared to spectrographic analyses of the plastic used as nest sites in the research and were found to have similar characteristics.

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INTRODUCTION

The Siamese fighting fish, Betta splendens Regan belongs to the order Labyrinthici, family Anabantidae. All members of this family possess a labyrinth which consists of a pair of vascularized cavities located in the sides of the head above the gills. The fish gulp air from the water surface into these cavities (Smith, 1945; Forselius, 1957). Since this species normally inhabits shallow, stagnant ponds, the labyrinth serves as a supplementary device for obtaining oxygen (Smith, 1937).

Within this family there is a diversity of reproductive behavior. Some species are bubble nest builders while others are oral incubators (Forselius, 1957). It is probable that bubble nest building was evolved from the habit of air breathing. Oral incubation as exhibited by Betta Pugnax Cantor, Betta brederi Myers and Betta picta Cuvier and Valenciennes has in turn evolved from the bubble nest building habit. Since Betta splendens is a bubble nest builder, it is considered to be rather primitive as regards reproductive behavior.

Normally the male of the species constructs the bubble nest and cares for the young although Forselius (1957) has reported that the female will also perform these functions.

A nest consists of a group of mucous-coated air bubbles deposited on the surface of the water. In nature these nests are constructed under floating objects such as leaves (Smith, 1945). The size and shape of the nest is usually determined by factors such as the availability of nesting material and the intensity of the nest building activity (Forselius, 1957). The nests in our laboratory were roughly circular in shape and varied in area from one cm^2 to 220 cm^2. They serve to protect the eggs and young (Breder and Rosen, 1966, Forselius, 1957) and also function as territory markers and to attract females to the territory (Braddock and Braddock, 1959, Forselius, 1957, and Picciolo, 1964).

In nature, the males will not begin to build nests in the presence of females (Smith, 1945) and will drive them away if the nest is not complete. In the laboratory, however, the presence of a female or a male actually causes an increase in nest building activity if the pair is physically, but not visually isolated. The fish will also construct nests when alone.

After mating, the male will gather the eggs in his mouth and deposit them in the nest by "blowing" them into the bubble mass. During the incubation period (30 to 40 hours), the male constantly repairs the nest by replacing burst bubbles. This replacement has two functions: to increase the firmness of the nest and to raise the eggs in the nest slightly closer to the surface where oxygen is more plantiful (Forselius, 1957).

After hatching, the male retrieves and replaces any young that have fallen out of the nest. After the free swimming stage is reached (three or four days), the nest is abandoned Breder and Rosen, 1966).

The Siamese fighting fish has been the subject of much popular and scientific literature. One of the earliest scientific studies was that of Regan (1909) who described the species. In 1932, Lissman discussed the ability of *Betta splendens* to discriminate colors and to react to various models. Smith (1937, 1945) presented information regarding the behavior and ecology of the species. Forselius (1957) in an extensive work described the nesting and reproductive behavior of this and other anabantid species. Braddock and Braddock (1955) discussed aggressive behavior in females of *Betta splendens* and (1959) the development of nesting behavior.

Many studies have been performed that involved conditioning. Lissman (1932) conditioned *Betta splendens* to respond to several colors. Otis and Cerf (1963) used conditioned avoidance learning to compare *Betta splendens* and the goldfish (*Carrasius auratus* Linneaus). Also in 1963, Adler and Hogan studied the extinction of an innate response via classical conditioning. Thompson (1963, 1966) and Thompson and Sturm (1965a, 1965b) utilized conditioning techniques to study various aspects of the fishes' behavior.

Studies involving conditioning have two major inherent weaknesses. Although animals can be trained to discriminate, they can also learn _not_ to discriminate. In addition, because the ability to learn varies greatly among individuals, one can seldom be sure of an adequate sample.

The following studies and also the present one, involve no conditioning, but take advantage of a known portion of the natural, and presumably instinctive, behavior of _Betta splendens_, namely, the building of bubble nests by the male. This method has two advantages when compared to the usual technique of measuring discriminatory ability, that of conditioning, in that quantitative data may be obtained in terms of the number of square centimeters of nest bubbles deposited under each particular form, and negative conditioning is eliminated by using individuals naive to the situation.

Braddock, Braddock, and Kowalk (1960) and Childs (1964) have established the range of area within which different sizes of discs can be distinguished by the fish are that these differences are distinguished as proportional rather than absolute. Braddock, Braddock and Richter (1961) and Cerny and Braddock (1965) have demonstrated that compact shapes are discriminated from elongate shapes and that discs are preferred to square or triangles. Gude (1965) has reported that yellow and green colors are preferred to red and blue and red is preferred to blue.

This study is an attempt to determine whether size, shape, and color have different values as stimuli in the nesting situation, and if so, the relative value of each. In testing, a plastic disc having a preferred size but of a non-preferred color, was matched with another plastic disc having the preferred color, but of a non-preferred size. Similar combinations were also made involving color and shape. It was then determined whether or not the stimulus of size overrode the stimulus of color or vice-versa and also whether the stimulus value of shape was greater than the stimulus value of color.

Size versus shape was not tested due to the exigencies of time. Also, it was hoped that the results obtained from the tests actually performed would provide clues to their relative values as stimuli. Thus, if color were preferred to size to a large degree and to shape in a small degree, shape should outrank size. If, on the other hand, color out-ranked size and shape outranked color, the stimulus hierarchy would be: shape, color, size in that order.

METHODS AND MATERIALS

Most of the fish used in these experiments were young males purchased at a local pet shop. Others were obtained from a private party in Livonia, Michigan. Fifty were purchased initially and of these, 20 were chosen as test fish, 10 as stimulus fish and the remaining 20 were kept in reserve in the event of the death of any of the former. A second group was later purchased to complete a second series of experiments. Of these, 20 were used as test fish, 10 as stimulus fish and the remaining 10 were kept in reserve. The size of all individuals ranged from 3.5 to 4 cm. standard length.

The experiments were carried out in a laboratory located on the third floor of the Natural Science building on the Michigan State University campus. Each test and reserve fish was separately housed in a one gallon, wide-mouthed jar, while each stimulus fish was kept in a stimulus aquarium which will be described later. The jars were filled to a depth of 12.5 cm. (approximately 2/3 full) and contained no gravel or plants. All waste products and uneaten food were siphoned out weekly. The mouths of the jars were covered with 5" x 5" squares of heavy gauge sheet metal to retard evaporation. The water used in the jars and aquaria was aged tap water, and, as evaporation occurred, the water lost was replaced by distilled water. The water in all of the aquaria was removed and replaced at the conclusion of each week's testing.

The fish were physically isolated from one another at all times, and, with the exception of the time spent in the test and stimulus aquaria, were also isolated visually by opaque plastic strips which were placed between the gallon jars.

The jars containing the test fish were kept on top of the test enclosure, while the jars containing the reserve fish were kept in double rows on a wooden rack.

The laboratory contained three windows which faced north. These windows were covered with shades which allowed little natural light to enter the laboratory thus keeping light conditions as constant as possible. The laboratory itself was lighted by sixteen 40-watt fluorescent bulbs which were controlled by an automatic timer to provide twelve hours of light from 8 A.M. to 8 P.M. and twelve hours of darkness.

Other environmental conditions were kept as constant as possible. The laboratory was heated with steam heat and also contained an air conditioner. A fan kept the air circulating and the temperature was maintained at 80-82^{o} F.

The fish were fed daily with finely ground commercial shrimp supplemented on alternate days with brine shrimp nauplii.

An enclosure 41 inches wide, 14 feet long, and five feet high contained tables on which the ten test aquaria were placed. Ten 30-watt fluorescent bulbs were located at 18 inch intervals (perpendicular to the long axis of the enclosure). Centered directly beneath and parallel to each bulb was a 20 gallon test aquarium filled to a depth of 18 cm. and its adjacent stimulus aquarium (Fig. 1). The bulbs were 18 inches from the surface of the water and remained lit 24 hours a day. The inside of the enclosure was painted with flat black enamel and had one removable side. The enclosure was light-tight when closed and allowed no outside light from the laboratory to reach the test situation.
A thermostatically controlled exhaust fan kept the temperature constant at $81^{\circ}F \pm 2^{\circ}$.

Each test aquarium measured 76 cm. in length, 33 cm. in width, and was 30.5 cm. deep. The bottom was covered with a layer of black gravel approximately one cm. deep. No vegetation was present. Each aquarium was externally covered with opaque, flat black plastic. An opening measuring 11.4 cm. square was cut in the right side of this black plastic five cm. from the bottom and equidistant from both ends.

The stimulus aquaria consisted of one-quart battery jars measuring 15 cm. in length, 15 cm. in height and 8.5 cm in width. These were covered externally on three sides by translucent, matte plastic. The open side was placed against the opening on the right side of the test aquarium. This

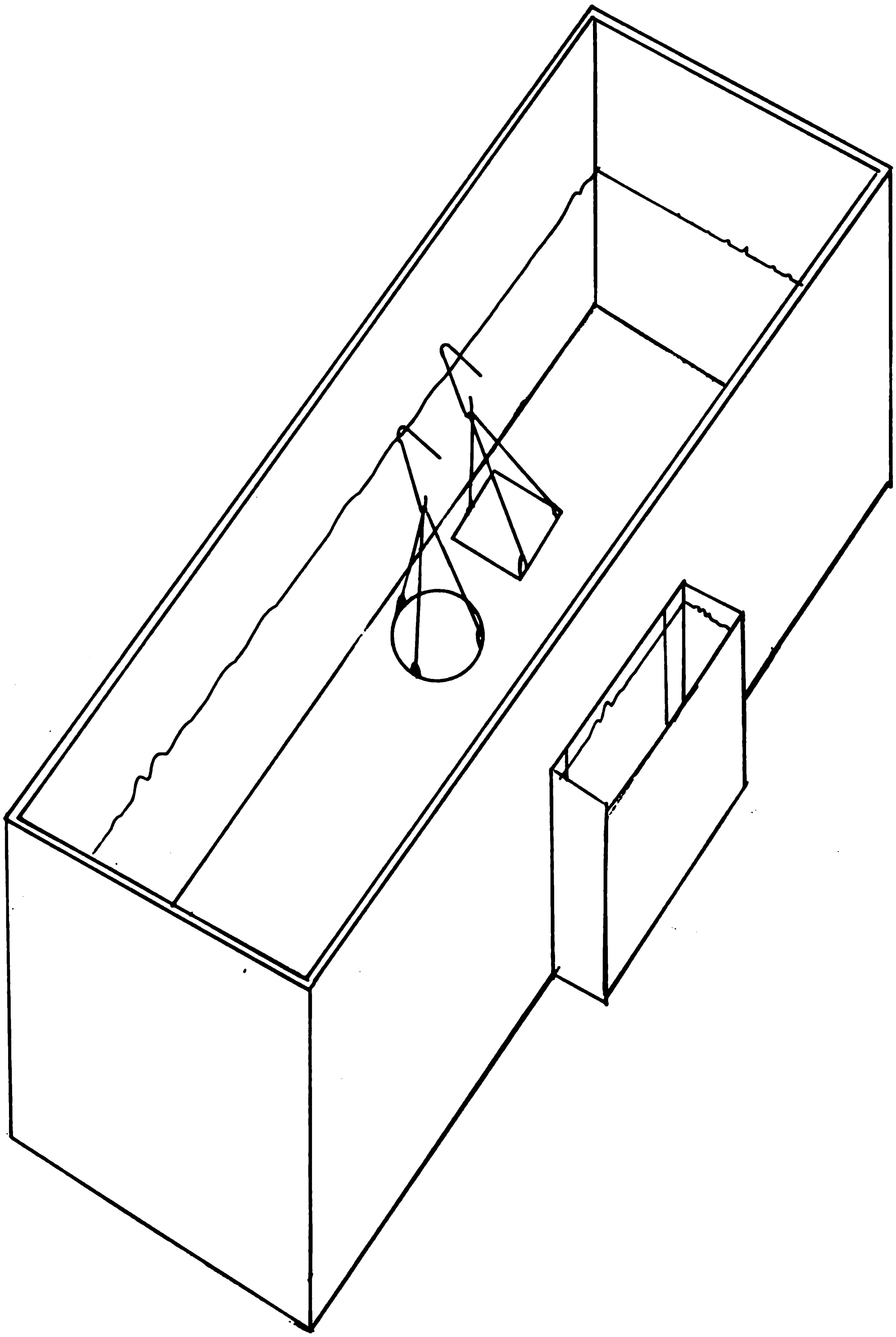

Figure 1. Test and Stimulus Aquaria.

allowed visual contact between the stimulus fish and the test fish.[1] The bottom of each stimulus aquarium was covered with approximately one cm. of black gravel and was filled with water so that its level corresponded with the water level in the test aquarium.

The various forms used as nest sites were suspended in the test aquaria in contact with the water surface and were constructed of 0.0001 inch matte laminating vinyl. Each form had three tabs which were bent perpendicuarly to the plane of the form. Each tab had a small hole in it through which a fine white thread was passed. The threads from the three tabs were then looped over a small hook constructed of copper wire which was fastened to the glass covering the top of the aquarium with Dow "Silastic" cement. These forms were floated at a distance of 9.5 cm. from either side and 2.5 cm. from the center of the aquarium (Fig. 2).

The colors used in these experiments were yellow, green, red, and blue. The desired color was obtained by placing a form of the particular color over the matte supporting form. The intensity of the color was regulated by placing white translucent and matte vinyl forms on top of the colored form. An intensity of two foot candles was maintained in all of the forms and colors. The numbers and colors of discs used

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[1]This promotes nesting activity since two males that can see one another and are unable to make physical contact will construct bubble nests possibly as a displacement reaction.

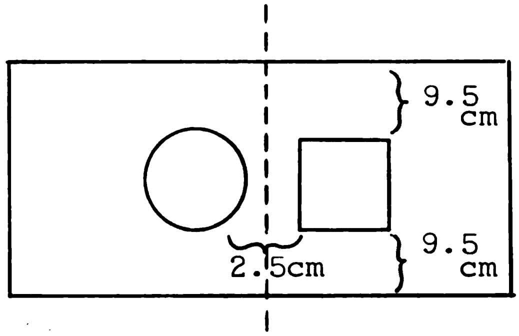

Figure 2. Positions of forms in aquaria.

to produce the two foot candle intensity are listed in the Appendix. The intensity was determined by measuring the amount of light transmitted through the forms with a Wesson model 756 light meter. The various colors were analyzed with a spectrophotometer to determine the percentage of light transmitted at the various wavelengths. This information is presented in detail in Table 1.

In the test situation, the only light reaching the floating forms came from the fluorescent bulbs located above each aquarium. All reflective surfaces in the enclosure were painted with flat black plastic, and the bottoms of the aquaria were covered with black gravel. Thus reflected light was minimized and the forms, therefore, emitted only transmitted light.

After a series of pilot studies during which techniques were tested and standardized, four series of experiments

Table 1. Spectrographic Analyses of Plastics

Color	Spectral Limits	Peak Intensity
Yellow	522-775 mμ	710 mμ
Green	498-584 mμ	548 mμ
Red	609-786 mμ	706 mμ
Blue	386-591 mμ	469 mμ

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

Color	Limits of Transmission
Yellow	575-585 mμ
Green	492-575 mμ
Red	647-723 mμ
Blue	424-455 mμ

were conducted. In the first, the relative stimulus values of size and color were compared. Two discs of unequal size were presented to the fish. The area of the larger disc was 275 cm^2 and the area of the smaller was 100 cm^2 (Fig. 3).

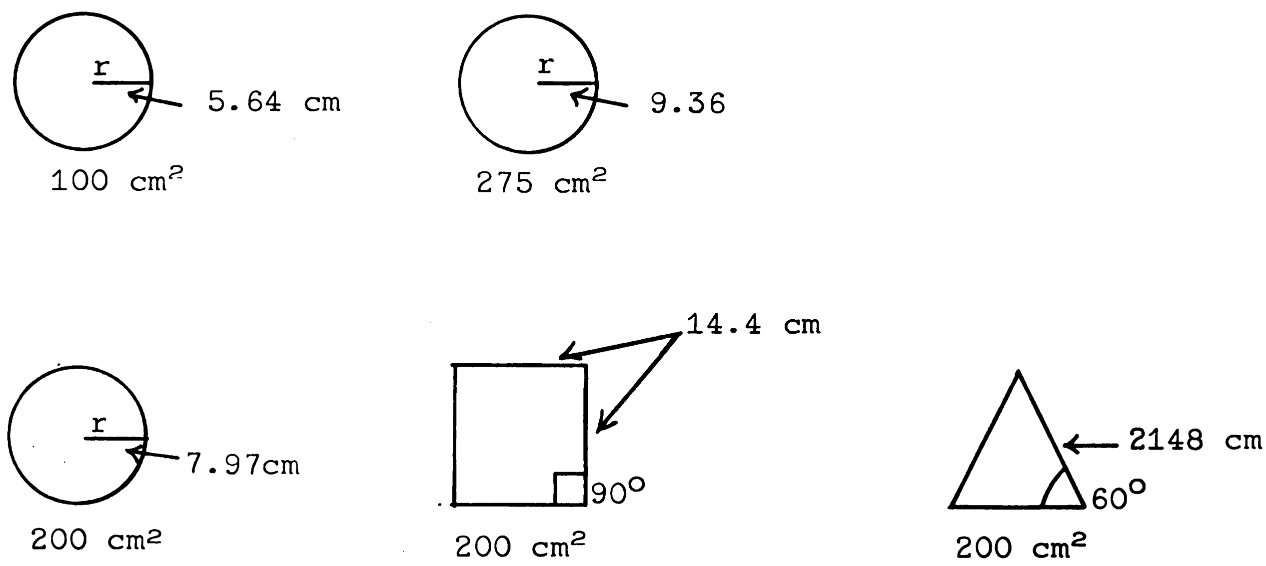

Figure 3. Dimensions of form.

This combination of disc sizes was chosen because, in an earlier work (Childs, 1964), it was found that the greatest size discrimination shown by the fish occurred when this particular combination of discs were used. All possible combinations of the large and small discs and the above mentioned colors were presented to the fish. These are illustrated in Figure 4.

Series 1		Series 2		Series 3		Series 4	
275 cm^2	100 cm^2	○	□	○	△	□	△
Y	G	Y	G	Y	G	Y	G
Y	R	Y	R	Y	R	Y	R
Y	B	Y	B	Y	B	Y	B
G	Y	G	Y	G	Y	G	Y
G	R	G	R	G	R	G	R
G	B	G	B	G	B	G	B
R	Y	R	Y	R	Y	R	Y
R	G	R	G	R	G	R	G
R	B	R	B	R	B	R	B
B	Y	B	Y	B	Y	B	Y
B	G	B	G	B	G	B	G
B	R	B	R	B	R	B	R

Y - yellow R - red

G - green B - blue

Figure 4. Color-form combinations.

The second, third and fourth series of experiments concerned the relative stimulus values of shape and color. Three shapes: circles, squares, and equilateral triangles, each with an area of 200 cm^2, were presented to the fish in various pair and color combinations (Fig. 4). This size was chosen because it was used in an earlier study (Cerny and Braddock, 1965) on shape discrimination and it was desired that testing conditions remain as uniform as possible. In this same study, it appeared that no preference exists between squares and equilateral triangles in the nesting situation. This, of course, does not prove that _B. splendens_ cannot distinguish the difference. By adding the color factor it was hoped that light might be shed on this matter. This species has also demonstrated a general lack of preference between yellow and green colors. If, for instance, a combination of square and green were strongly preferred over triangle and yellow, it could then be inferred that the two shapes are discriminated. Again, all possible combinations of shape and color were utilized (Fig. 4).

A coin was flipped to determine which side of the aquarium a particular form should occupy. Each fish was assigned an identification number and later a random number from a table. The identification numbers of the fish were written on slips of paper and drawn out of a container. The first ten fish drawn were tested on the first day and the second ten on the second day. Thus, every other day a particular fish was presented with a different situation with a one day rest period between tests. Each test situation involved 20 different fish. The random numbers were arranged in order of increasing magnitude and each fish was placed according to its corresponding random number. The first individual was placed in aquarium "A", the second fish in aquarium "B" and so on.

Before being placed in the test situation, the fish were fed finely ground commercial shrimp or brine shrimp nauplii on alternate days. The barometric pressure was noted and recorded. This was done since a third phase of the experiments involved an attempt to correlate barometric pressure and nest building activity. The water temperature of the bottles and test aquaria was recorded, and, if necessary, the temperatures were equalized by placing hot or cold wet paper towels around the jars. The fish were then netted and placed in the test aquaria. This was done in such a manner as to create a minimum of disturbance and usually required no more than five seconds. The fish were always introduced at the north end of the aquaria since this was the removable side of the test enclosure. The tops of the aquaria were covered with clear window glass and the side of the enclosure was put in place and thus made light-tight. The fish remained in the test situation for 24 hours. At the end of this period the barometric pressure was recorded and the amount of bubbles deposited under each form was noted and recorded. The bubbles were always measured in square centimeters. A mirror was used to observe the bubbles on the underside of the forms and the amount deposited was estimated by determining the proportion of the particular form covered. The water temperature of the jars and aquaria were again taken and any adjustments, if necessary, were made. The fish were then returned to their respective jars and fed. The bubbles were removed from the forms, thus preparing the aquaria for the next set of observations.

Preparation for this research began in September, 1965. Plastic for the floating forms was purchased and prepared. Some new aquaria were purchased and others were repaired. The enclosure was readied and the young fish were fed twice daily to increase their size to a standard length greater than 3.5 cm which was felt to be an adequate size for testing. The actual tests were conducted daily, Monday through Saturday, from July 11 to November 20, 1966 and from May 29 to July 31, 1967.

RESULTS

The experiments reported here may be divided into four separate series. Each of these involved 12 different pairings of forms differing in size and color or shape and color (Fig. 4). Preference was measured by the amount of bubbles deposited under a particular form. Thus, if a fish deposited more bubbles under one form than another, it was said to have a preference for that form.

The data from the experiments were statistically analyzed using the method of matched observations[1] (mean of the differences significantly different from zero). When the mean of the differences in the areas of the bubbles deposited under each form was found to be significantly different from zero at at least the 5% level, it was taken as an indication that a preference existed.

During the course of the experiments, 11 fish died. None died during a test period. They were replaced by fish that had been purchased at the same time as the deceased and had been maintained in an environment identical to that of the test fish.

When the experimental data were being analyzed, those fish that deposited no bubbles under either form were omitted,

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[1]Norman T. J. Bailey, Statistical Methods in Biology (New York: John Wiley and Sons, Inc., 1959), pp. 44-47.

since only positive responses were being measured and those fish that gave no response did not contribute any data.

It was determined in a previous work that no preference is shown for the right or left hand position of the form in the test aquaria (Gude, 1965).

It should be kept in mind that previous work has established that Betta splendens prefer the larger of two discs (Braddock, Braddock, and Kowalk, 1960 and Childs, 1964); prefer circles to squares and triangles (Braddock, Braddock, and Richter, 1961 and Braddock and Cerny, 1965); and prefer yellow and green to red and blue and red to blue (Gude, 1965).

In the first series of experiments the fish were presented with pairings of circles of various sizes and colors (Fig. 4). The results appear in Table 2.

Under the heading "275 cm^2 circle" the colors have been arranged in the order of preference (i.e., yellow, green, red, and blue) as has been shown in a previous work (Gude, 1965). In the "100 cm^2 circle" (various permutations of yellow, red and blue) have been arranged so as to be different from the larger circle and still be in the order of decreasing preference by the fish. The quantity of bubbles deposited under each particular disc has been listed in square centimeters. The "N" column represents the number of fish out of a population of 20 that deposited bubbles under either disc. The differences in the amount of bubbles deposited under each disc of a particular set is listed in the "d" column.

Table 2. Circles

Set	275 cm^2 Circle	Bubbles deposited (cm^2)	100 cm^2 Circle	Bubbles deposited (cm^2)	N	d(cm^2)	t
1	Y#	250	G	55	15	+195	2.45*
2	Y#	335	R	25	12	+310	4.71****
3	Y#	520	B	55	15	+465	5.21****
4	G	185	Y	275	13	-90	0.652
5	G#	315	R	0	11	+315	6.99****
6	G#	420	B	0	12	+420	7.26****
7	R	35	Y#	325	13	-290	3.75***
8	R	90	G	300	13	-210	2.11
9	R	255	B	148	13	+107	1.33
10	B	285	Y	245	14	+40	0.295
11	B	180	G#	480	13	-300	2.73**
12	B#	530	R	70	14	+460	8.22****

- favored disc

* - significant at the 5% (.05) level

** - significant at the 2% (.02) level

*** - significant at the 1% (.01) level

**** - significant at the .1% (.001) level

The larger (275 cm^2) disc was always considered as being "+". Thus a number preceded by a "+" was that excess amount of bubbles in square centimeters deposited under the larger disc. The "t" value, obtained from statistically analyzing each set, has been listed in the "t" column. The significance of each "t" value, is denoted by the asterisk(s) following it.

In the first set of series 1, the larger yellow disc was preferred over the smaller green disc.

The second set of the first series matched a 275 cm^2 yellow disc and a 100 cm^2 red disc. The larger yellow disc was preferred.

In the third set of series 1, a larger yellow disc was paired with a smaller blue disc. The larger yellow disc was again preferred.

A larger green disc and a smaller yellow disc made up the fourth set. Here, no definite preference was shown for either one.

The fifth set of discs of this series consisted of a larger green disc and a smaller red disc. As can be seen from Table 2, a very definite preference was shown for the green disc, with no bubbles at all deposited under the smaller red disc.

The sixth set of series 1 matched a larger green with a smaller blue disc. As in the preceding set, a definite preference was shown for the larger green disc and again no bubbles were deposited under the smaller disc of this set (blue).

In the seventh set, a larger red disc was paired with a smaller yellow disc. The larger disc was of a preferred size but of a non-preferred color and the smaller disc was of a non-preferred size but of a preferred color. In this case, the smaller yellow disc was the one preferred.

A larger red disc and a smaller green one made up the eighth set. In this case no preference was shown for either disc.

When the raw data for set number 8 was checked, it was found that only two fish deposited bubbles under the 275 cm^2 red disc and of these, one fish deposited 70 cm^2. When the data from the other sets was checked, it was noted that this same fish deposited bubbles under the "wrong" disc seven times out of twelve and always in large amounts ranging from 50 cm^2 to 80 cm^2. It would thus appear that this fish is somewhat aberrant in its behavior and because of this, it caused a shift in the significance of the results. Only in this eighth set of discs, however, could the lack of significance be attributed solely to this particular fish. When the results from this combination were re-analyzed omitting this fish, the "t" value of 8.02 was significant at the 0.1% (0.001) level. Because of the consistent aberrancy of this fish's behavior it was felt that it could be eliminated from this particular set thereby making the remaining data significant and thus indicating that the fish showed a definite preference for the smaller green disc.

The fish was not eliminated from the other disc sets since this would only have increased the significance and since the significance was already at at least the 5% (.05) level, it was felt that this was not necessary.

The ninth set was composed of a larger red disc and a smaller blue disc. Although there were almost twice as many bubbles deposited under the larger red disc as under the smaller blue disc, this difference was not significant at the accepted level of 5% (.05) and it must then be tentatively inferred that there was no preference shown for either one.

The tenth set of discs matched a larger blue disc with a smaller yellow disc. As evidenced by the "t" value of .295, there was no significant difference in the amount of bubbles deposited under them.

In the eleventh set a larger blue disc was paired with a smaller green disc. As shown in Table 2, the fish showed a definite preference for the smaller green disc.

The twelfth and last set of this series was composed of a larger blue disc and a smaller red disc. As is apparent from the highly significant "t" value of this set, the fish showed a very definite preference for the larger blue disc.

In concluding this series it may be stated that yellow was preferred over green, and yellow and green are preferred over red and blue regardless of the size of the matching disc. This was observed in eight of the 12 disc combinations. No evidence was found that would indicate the fish prefer red over blue.

In this second series of experiments, the fish were presented with different color combinations of circles and squares (Fig. 4), having the same area (200 cm^2). The results are tabulated in the same manner as the first series and are presented in Table 3.

The first set of the second series matched a yellow circle with a green square. Here a preference was shown for the yellow circle.

In the second set a yellow circle was paired with a red square. A definite preference was shown by the fish for the yellow circle.

A yellow circle and a blue square comprised the third set. In this instance the yellow circle was again preferred.

The fourth set was composed of a green circle and a yellow square. No preference was shown for either form.

Table 3. Circles and Squares

Set	Circle	Cm^2 Bubbles	Square	Cm^2 Bubbles	N	$d(cm^2)$	t
1	Y#	525	G	90	15	435	7.85****
2	Y#	575	R	0	13	575	3.38***
3	Y#	470	B	140	18	330	2.46*
4	G	175	Y	405	16	230	1.73
5	G#	595	R	50	14	545	4.76****
6	G#	480	B	50	17	430	5.80****
7	R	0	Y#	695	16	-695	5.97****
8	R	45	G#	450	15	-405	3.87***
9	R	195	B	115	12	80	0.879
10	B	35	Y#	350	12	-315	2.93**
11	B	25	G#	440	15	415	4.74****
12	B	255	R	85	13	170	1.89

- form preferred

* - significant at the 5% (.05) level

** - significant at the 2% (.02) level

*** - significant at the 1% (.01) level

**** - significant at the 0.1% (0.001) level

The fifth set matched a green circle with a red square. The "t" value of 4.76 indicates that the fish showed a definite preference for the green circle.

In the sixth set a green circle was paired with a blue square. As before, the green circle was preferred to the blue square.

In sets seven and eight, red discs were paired with yellow squares and green squares respectively. In neither set was the red disc preferred.

Set nine consisted of a red circle and a blue square. In this set no preference was shown for either disc.

The tenth and eleventh sets were composed of blue circles matched with yellow and green squares respectively. In both sets the squares were preferred over the circles.

The last set in this series matched a blue circle with a red square. There was no preference shown for either form of this set.

In conclusion it may be stated that the fish generally demonstrated a preference for the colors yellow over green and yellow and green over red and blue, regardless of the shape of the floating form. Again, no evidence was found for preference of red over blue.

The third series was concerned with the preference shown when the fish were presented with circles and triangles of various color combinations (Fig. 4). Table 4 presents the results from this set and is tabulated in the same manner as Tables 2 and 3.

Table 4. Circles and Triangles.

Set	Circle	Cm^2 Bubbles	Triangle	Cm^2 Bubbles	N	$d(cm^2)$	t
1	Y	365	G	295	15	+70	0.296
2	Y#	565	R	45	12	+520	3.56***
3	Y#	1105	B	240	18	+865	4.02****
4	G	480	Y	210	15	+270	1.55
5	G#	505	R	10	14	+495	6.03****
6	G#	650	B	65	17	+585	4.64****
7	R	0	Y#	620	13	-620	4.16***
8	R	95	G#	590	16	-495	2.32*
9	R	595	B	220	11	+345	1.17
10	B	245	Y	415	13	-170	1.11
11	B	50	G#	655	16	-605	5.58****
12	B	210	R	160	6	+50	0.391

- form preferred

* - significant at the 5% (.05) level

** - significant at the 2% (.02) level

*** - significant at the 1% (.01) level

**** - significant at the 0.1% (.001) level

In the first set of this series the fish were given a yellow circle and a green triangle. The difference in the amounts of bubbles deposited under each form was not significant thus indicating no preference for either one.

Sets two and three matched yellow circles with red and blue triangles respectively. In both instances the difference in the amounts of bubbles deposited under the yellow circle and the matching triangle was highly significant in favor of the yellow circles indicating that the fish were showing a definite preference for the circles.

The fourth set paired a green circle with a yellow triangle. Although the fish deposited more than twice as many bubbles under the green circle as under the yellow triangle, the difference in the amounts was not statistically significant thus it must be said that no evidence exists for preference for either form.

In sets five and six, green circles were paired with a red and a blue triangle respectively. In both instances a definite preference was shown for the green circles.

Red circles were paired with a yellow and a green triangle in sets seven and eight. The red circles were given no preference in either case.

The ninth set consisted of a red circle and a blue triangle. In this case the difference in the amounts of bubbles deposited under each form was not significant. Thus, no preference was indicated for either form.

Set number 10 matched a blue circle with a yellow triangle. As in the previous set, no preference was shown for either form.

In the eleventh set, a blue circle was paired with a green triangle. The fish showed a definite preference for the green triangle as evidenced by the highly significant "t" value.

The twelfth and last set of this series consisted of a blue circle and a red triangle. No preference was shown by the fish for either form.

It may be concluded that the colors yellow and green are preferred over red and blue, as in the previous series of experiments regardless of the shape of the matching form. No evidence was found for any preference for yellow over green or red over blue.

In the fourth and last series of experiments, the fish were presented with squares and triangles of various color combinations (Fig. 4). Table 5 presents the results from this series and is tabulated in the same manner as Tables 2, 3, and 4.

The first set of this series matched a yellow square with a green triangle. The fish seemed to show a preference for the yellow square since 615 cm^2 of bubbles were deposited under it and only 230 cm^2 were deposited under the green triangle. The difference in these amounts was not statistically significant, however, and it must be concluded tentatively that the fish did not show any preference.

Table 5. Squares and Triangles

Set	Square	Cm^2 Bubbles	Triangle	Cm^2 Bubbles	N	$d(cm^2)$	t
1	Y	615	G	230	16	+385	1.92
2	Y#	765	R	0	12	+765	4.96****
3	Y#	965	B	355	20	+620	2.74**
4	G	240	Y	690	16	-450	2.02
5	G#	550	R	120	15	+530	3.19***
6	G#	565	B	0	14	+565	5.69****
7	R	50	Y#	540	13	-490	4.23***
8	R	70	G#	480	17	-410	5.08****
9	R	285	B	650	16	-365	1.89
10	B	100	Y#	560	18	-460	6.95****
11	B	70	G#	520	16	-450	5.39****
12	B	180	R	210	15	-30	0.317

- shape preferred

* - significant at the 5% (.05) level

** - significant at the 2% (.02) level

*** - significant at the 1% (.01) level

**** - significant at the .1% (.001) level

In the second set a yellow square was paired with a red triangle. Here a very definite preference was shown for the yellow square in that 765 cm^2 of bubbles were deposited under it, while none were placed under the red triangle.

The third set was composed of a yellow square and a blue triangle. As in the previous set, the yellow square was preferred.

The fourth set matched a green square with a yellow triangle. No preference was shown for either, since the difference in the amounts of bubbles deposited under each form was not significant.

Green squares paired with a red and a blue triangle made up the fifth and sixth sets respectively. In both cases, the fish showed a definite preference for the green squares.

In the seventh and eighth sets, red squares were matched with a yellow triangle and a green triangle respectively. In both instances, the triangles were preferred.

The ninth set of this series paired a red square with a blue triangle. Statistically, the fish showed no preference for either form although they deposited a total of 650 cm^2 of bubbles under the blue triangle and only 285 cm^2 under the red square.

The tenth and eleventh sets were composed of blue squares matched with yellow and green triangles respectively. The blue squares were given no preference in either case.

The last set was made up of a blue square and a red triangle. The fish showed no significant preference for either form of this series.

As in the previous series, it may be concluded that the fish generally preferred yellow and green over red and blue regardless of the shape of the form. Again, no preference was shown for yellow over green or red over blue.

DISCUSSION

Many studies involving discrimination by animals have been carried out. The species studied have varied greatly and include representatives from all classes of vertebrates and some invertebrates. Sutherland (1957a, 1960), Sutherland and Carr (1963) and Sutherland, Mackintosh and Mackintosh (1963) have performed extensive discrimination studies using Octopus vulgaris (Lamarck). Rensch (1957) studied the discriminatory ability of the Indian elephant and Dodwell (1957) studied the ability of hooded rats to distinguish various shapes. Many discrimination studies have been conducted using birds as the test animal. One of the better known is Tinbergen's work with the European Blackbird (Turdus merula, L.) (1958). In 1911, Casteel studied the discriminative ability of the painted turtle.

Although fish have not been studied with respect to their discriminatory abilities to the extent certain other animals have, many instances may be cited. Hemmings and Mathews (1963) and Mathews (1964) have studied shape discrimination in tropical fish. The discrimination of angles and patterns by the goldfish has been studied by Rowley (1934) and Mackintosh and Sutherland (1963).

The above-mentioned studies all have one aspect in common: conditioning techniques were employed.

The disadvantages of conditioning techniques were mentioned in the introductory section of this paper and the intent was to avoid these in the present work.

Although some studies of *Betta splendens* have involved conditioning (Goldstein, 1965, 1967; Thompson, 1963, 1966; and Thompson and Sturm, 1965a, 1965b) the present study utilizes a phase of the natural behavior of the animal and involves no known conditioning.

It is known that *Betta splendens* prefers the larger of two discs (Childs, 1964); prefers circles to squares and triangles (Cerny and Braddock, 1965), and shows preference for yellow and green over red and blue and red over blue (Gude, 1965). In the present study, the fish were given various combinations of sizes, shapes, and colors (Fig. 4) and it was determined which aspects of these factors had the greatest stimulus value for the fish with respect to being chosen as a nest site.

Color Preferences

Table 6 summarizes the results of Tables 2 through 5. An entry in this table gives the preferred color and the significance level of that preference for a given combination of two colors and two size or shape forms. Thus, the first column represents the preferences shown by fish for colors when one color was presented as a large circle and the other color as a small circle. The other columns list the preferences for two forms of similar area but different shape.

The rows of Table 6 list the six possible combinations of four colors taken two at a time. Thus, the first row lists the yellow and green combination, the second row the yellow and red combination, and so forth. The upper entry in each row lists the color of item 1 first, and the color of item 2 second, while the lower entry in each row lists the reciprocal test. Thus, the first row lists those tests in which item 1 was yellow in the upper part of each entry. The entry Y*/Y in the upper left hand corner of the table indicates that when a large circle was yellow and a small circle green, the fish blew more bubbles under the yellow circle, and that the difference was significant at the 5% level (Y*). The lower entry indicates that when a large green circle was paired with a small yellow circle, the yellow circle had more bubbles blown under it but that the difference was not statistically significant (Y).

The first row of Table 6 indicates that in all but one set (green circle-yellow triangle), the fish showed a preference for the yellow forms over the green regardless of their shape or size. Although there are only two individual sets where statistically significant preferences were shown for the yellow form, when the results from all the combinations involving yellow and green were pooled and subjected to statistical analysis, they were found to be significant at the .1% (.001) level indicating a definite preference for yellow over green. This finding lends support to Gude's

Table 6. Comparison of Color Preferences in Reciprocal Tests

Combinations (1) / (2)		(1) / (2)	○ / ∘	○ / □	○ / △	□ / △
Y / G	G / Y		Y* / Y	Y**** / Y	Y / G	Y / Y
Y / R	R / Y		Y**** / Y***	Y**** / Y****	Y**** / Y***	Y**** / Y***
Y / B	B / Y		Y**** / B	Y* / Y**	Y**** / Y	Y** / Y****
G / R	R / G		G**** / G	G**** / G***	G**** / G*	G*** / G****
G / B	B / G		G**** / G**	G**** / G****	G**** / G****	G**** / G****
R / B	B / R		R / B****	R / B	R / B	B / R

Letter indicates color of form majority of bubbles were deposited under.
Asterisk(s) indicate significance:
* significant at 5% (.05) level
** significant at 2% (.02) level
*** significant at 1% (.01) level
**** significant at .1% (.001) level

(1965) conclusion that the fish exhibit a "subtle" preference for yellow over green.

The second through the fifth rows of Table 6 indicate that the fish showed a preference for yellow and green over red and blue. No statistical tests were performed on these sets as a unit since in only three (large blue circle-small yellow circle, blue circle-yellow triangle, and large red circle-small green circle) out of a total of 32 sets was there no significant preference shown for either the yellow or green forms when paired with either red or blue forms. The amounts of bubbles deposited under either the yellow or the green forms of the remaining 29 sets were significantly greater than the amounts deposited under the matching red or blue forms indicating a definite preference for yellow and green over red and blue.

The last row of Table 6 contains the red-blue, and blue-red combinations. It will be noted that the large circle of series one, the circles from series two and three and the triangle of series four were consistently chosen by the fish regardless of their color. It thus appears that the fish were discriminating on the basis of size and shape rather than color and in fact did not discriminate at all between the colors red and blue. These results are in opposition to those found by Gude who stated that the fish show a significant preference for red over blue.

Gude suggested an explanation for the Fishes' preference for yellow and green over red and blue. He stated that the fish show preferences for those colors that are transmitted by the leaves of the plants which occur in their natural environment. Several genera of aquatic plants native to Thailand were subjected to spectrographic analysis. The wavelengths of the peak intensities of the plant pigments were found to coincide closely with the peak intensities transmitted by the yellow and green plastic used in this research. Thus, the yellow and green plastic may seem more "natural" to the fish than the red and blue plastic.

It was also found that the entire spectral range of the yellow plastic included both peak intensities shown by all plants analyzed, whereas the spectral range of the green plastic included merely the first peak intensity transmitted by only three of the five plant genera analyzed. This may explain the preference that the fish show for yellow over green.

Several explanations may be given for the fishes' apparent lack of preference between the red and the blue forms.

Gude found that the lower end of the spectrum transmitted by the red plastic barely overlaps the upper limit of the spectrum transmitted by the plants native to the fishes' habitat. On the other hand, the upper limit of the spectrum transmitted by the blue plastic barely overlaps the lower limit of the spectrum transmitted by the native plants. Thus, it is seen that neither color is in the "preferred range" and since both colors are considered as being undesirable (or at least "less desirable"), the fish do not discriminate between them.

A second hypothesis may be proposed. Gude analyzed sunlight with a spectrophotometer and found that the spectral limits of the blue discs contained the peak intensities of light emitted from blue sky. He postulated that the presence of such a transmission spectrum would indicate a complete absence of a suitable nest site. This idea may be valid to a certain extent; however, there are certain limitations. The spectral limits of the sky would be changed after being transmitted through the water. Other factors that cast doubt upon this hypothesis would be clouds, overhanging tree branches and leaves, and debris floating on the water.

A third explanation was given by Gude for the fishes' lack of preference for red or blue. He stated the fish are responding in a negative manner to particular wavelengths of light. Since the colors red, blue, and blue-green are those most often displayed during periods of aggressive behavior, they are the colors the fish will tend to avoid. He analyzed the light reflected from the tail fins of blue and blue-green fish (he was unable to analyze red) and found that the spectral limits of the blue plastic included both peak intensities of the reflected light from the blue and blue-green fish. Thus, the fish would tend to deposit fewer bubbles or even to avoid entirely a prospective nest site, which in this case would be the blue plastic forms, that transmitted particular wavelengths of light corresponding to aggressive body coloration.

From the preceding discussion it may be concluded that the fish prefer yellow to green, yellow and green to red or blue, and show no preference for either red or blue. It is still not known whether the fish do not discriminate between red and blue, or are simply failing to respond to either color when presented with this particular combination.

In tabulating the data, only those fish that gave a positive response were recorded. It is possible to determine the number of fish that did not blow bubbles under the various size, shape, and color combinations, especially those combinations involving red and blue, to determine if Gude's hypothesis was correct, namely that the fish were actually avoiding certain colors.

A chi-square analysis was performed on the data to determine the extent of randomness with respect to the number of fish depositing bubbles under each color combination. These results are presented in Table 7. The number of fish depositing bubbles under a particular color combination was recorded along with the number of times no bubbles were deposited under either form. These results give a chi-squared

Table 7. Statistical Analysis to Determine the Randomness of the Frequency of Nest Building

	Number of fish depositing bubbles	Number of fish depositing no bubbles	Total	Percent of total fish not depositing bubbles
Y G / G Y	121	39	160	24
Y R / R Y	104	56	160	35
Y B / B Y	128	32	160	20
G R / R G	115	45	160	28
G B / B G	120	40	160	25
R B / B R	100	60	160	38
	688	272	960	

value of 16.97 (5 degress of freedom) which is significant at the 1% (.01) level indicating that the number of fish depositing bubbles under each color combination was not equal. The red-blue and blue-red combinations made the greatest contribution to the chi-squared value because of the large proportion of the fish that blew no bubbles under either form. It thus appears that the fish are in fact avoiding those combinations containing only red and blue and this constitutes evidence for Gude's hypothesis.

Size and Shape as Modifiers of Color Preference

Because of the lack of a red-blue preference, the bottom row of colors in Table 6 will be omitted from this discussion.

It will be noted in the first column of Table 6 that in all cases where the large circle was also of a preferred color, it was shown significant preference by the fish. The significance of the preference was less or completely lacking when the preferred color was not present in the larger circle although in four of the five sets the fish still chose the color rather than the larger circle. The one exception was a non-significant preference for a large blue circle over a small yellow circle.

It thus appears that size can either interfere with or reinforce preferences shown by the fish. That this interaction was observed in all five sets, indicates that size is quite important with respect to modifying the fishes' preference.

The second column of Table 6 contains those color combinations involving circles and squares. The color preferences exhibited by the fish seemed to be affected by shape in only two out of the five sets. In the yellow-green and green-yellow combinations, the yellow form was chosen on both occasions. The significance of the preference, however, dropped from the .1% (.001) level when the circle was yellow to no significance when the yellow form was the square. The second set of combinations showing the effect of shape as a modifier of color preference was the green-red and red-green combinations. The effect in this case, however, was much less evident than in the first since the significance of the preferences shown for the green form dropped from the .1% (.001) level when the green form was the circle, to only the 1% (.01) level when the green form was the square.

The circle-triangle combinations are presented in the third column of Table 6. The effect of shape as a modifier of color preference appears in four of the five sets. In only one set (green-blue, blue-green combinations) was there no effect. In the first set of combinations it seems that preference for shape has completely overridden the (yellow-green, green-yellow) color preference since the circle was chosen both times regardless of its color. In the remaining three cases, the significance of the preference shown for the preferred color was less or completely lacking when the preferred color was not also on a preferred shape.

The last column of Table 6 presents the preferences shown for the square-triangle combinations. In only one set did the effect of shape appear to modify a color preference. A slight drop in significance from the .1% (.001) level when the yellow form was the square, to the 1% (.01) level when the yellow form was the triangle, occurred in the yellow-red and red-yellow combinations. It may thus be concluded that color preference by the fish is little modified by shape in the case of the square-triangle combinations.

In general, it may be stated that color is more important as an indicator of suitability of nesting sites than is size or shape. This is shown by Table 6 which illustrates that in most cases, regardless of the size or shape, a color preference, although at times not significant, is shown in the order yellow over green, yellow and green over red or blue, and no preference for red or blue.

That these color preferences may be modified by size and shape, has been discussed. It was found that size has a great effect on the fishes' choice of color since in five out of five sets, the choice was affected by the size of the larger disc. Shape appeared to have a slightly smaller effect than did size in the case of the circle-triangle combinations (four out of five sets showed the effect), an even lesser effect in the circle-square combinations (two sets out of five showing the effect) and in the square-triangle combinations (only one set out of the five showed any evidence of shape modifying the color preference).

That the circle is important to the fish can be inferred from Smith's statement (1945) that the fish normally deposit their bubble nests under round floating leaves. It is perhaps due to this that they prefer the circle in the test situation.

The size of the nest site would be important since if one was chosen that was too small, it might result in the loss of all, or part of a brood. Choice of a leaf that might be too large would be less critical since the fish would utilize only the necessary amount of area for its bubble nest. Shape would be important also, since if a fish chose a non-compact site, e.g., a long narrow leaf, it would tend to be less stable and more prone to disturbance than if a more compact site, e.g., a round leaf, were chosen.

Neither of the above factors, however, are as important as color. It has been previously stated that the fish normally choose a yellow or green nest site over red or blue regardless of its shape. In nature, if they chose a green or yellowish leaf, this leaf would be more apt to be in a permanent position, since these colors would indicate that it is alive, and would not tend to sink or be displaced by wind, etc. Also a live leaf would tend to benefit the eggs and fry which remain in the nest for several days, since it is an important producer of oxygen.

SUMMARY

1. Males of *Betta splendens* were found to discriminate between various colors and shapes and to show preference for certain of them.

2. The fish generally preferred color over shape.

3. The fish showed a preference for yellow over green.

4. The colors yellow and green were preferred over red and blue.

5. There was no evidence found for a preference of red over blue.

6. Size and shape appears to interfere with or reinforce the color preferences of the fish.

7. It was concluded that *Betta splendens* show preferences for those colors corresponding to the color of the leaves normally used as nest sites in their natural habitat.

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APPENDIX

Number of Forms Used to Produce Two Foot Candle Intensity*

Color	Number of Colored Forms	Number of Matte Forms
Y	3	1 white form
G	2	3 matte forms
R	1	6 matte forms
B	2	4 matte forms

*In addition to the supporting matte surface forms.