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FACTORS INFLUENCING THE SPAWNING FREQUENCY IN THE FEMALE CICHLID FISH *TILAPIA MACROCEPHALA*¹

BY LESTER R. ARONSON²

In a previous experiment on the influence of sexual exteroceptive factors on the ovulatory cycle (Aronson, 1945) it was demonstrated that while many completely isolated *Tilapia* females continue to spawn at infrequent intervals, the sight of a male markedly increases this oviposition frequency. Actual contact of male and female does not appear to be important, but chemical or vibratory stimuli may possibly increase the oviposition rate slightly. In the present undertaking, attempts have been made to analyze this visual stimulus. *Tilapia* pairs normally execute elaborate courtship and pre-spawning behavior patterns which are qualitatively similar for the two sexes, but quantitative records indicate marked differences in the frequency of occurrence of these behavioral items (Aronson, 1949). The next step is to find whether or not these quantitative differences in sexual behavior would be reflected in the relative stimulus values of male and female as regards spawning frequencies. It is known that in most vertebrates castration will reduce or abolish a considerable portion of sexual behavior (Beach, 1947). Hence it was also of interest to

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² Department of Animal Behavior, the American Museum of Natural History.

measure the stimulus values of castrated males and females, although because of the nature of the experiment and the technical difficulties involved, we were unable at this time to determine the effects of the gonadectomies on the behavior itself.

MATERIALS AND METHODS

Several aquaria, each 60 by 30 by 30 cm. and with a capacity of 54 liters, were divided in half by transparent glass partitions sealed into the tanks with aquarium cement. With these divided tanks, five experimental conditions were established: (A) male and female on opposite sides of the glass partition (control); (B) a female on either side of the glass partition; (C) a castrated male opposite an intact female; (D) a castrated female opposite an intact female; (E) a female completely isolated in a 26-liter aquarium, 35 by 30 by 25 cm., which contained only half as much water as any one of the other aquaria. This last condition may be considered a low-limit control for the frequency of spawning. These five experimental situations together will be referred to henceforth as a "group." Each of these conditions was duplicated 15 times, and thus there were 15 groups. All of the tanks were situated in a greenhouse the temperature of which was maintained at approximately 27° C. at all times. The outside walls of all aquaria were covered with a heavy coat of pale blue paint, but inspection holes, covered with cardboard flaps, were placed in the front walls. Each partitioned half of the 54-liter tanks and all the 26-liter low-limit control tanks were furnished with charcoal filters which were cleaned twice weekly.

SURGICAL PROCEDURES

Males and females were anesthetized by immersion in 300 cc. of a 3 per cent solution of urethane, made up just prior to the operations. As soon as the fish became sufficiently immobilized (about three to six minutes), it was removed from the urethane and placed in a finger bowl on a piece of cotton soaked in aquarium water. Considerable care was exercised throughout the operation to insure that respiration was not interrupted for any length of time. A small pool of water sufficient for respiration could be maintained around the mouth of the fish by pressing down on the wet cotton. Although it was possible to deepen the state of anesthesia at any time by another immersion of the fish in the urethane solution, it was generally found advantageous to work

at a sufficiently rapid rate so that as the operation neared completion the fish had already partly recovered from the effects of the anesthesia.

With the sharp point of a pair of iridectomy scissors, a small incision was made through the skin, scales, and body musculature into the peritoneal cavity at the point indicated by A in figure 1. With a heavier pair of scissors the incision was then extended anteriorly just ventral to the dorsal wall of the peritoneal cavity to the level of the pectoral girdle at point B (fig. 1). The incision

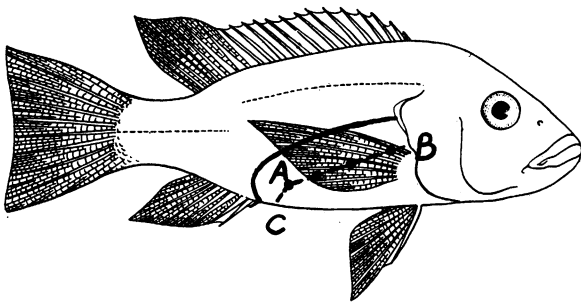


FIG. 1. Diagram showing location of incision (solid line) used in castrating males and females. Broken line represents position of dorsal wall of peritoneum. Dots on incision line represent sutures. First incision, A to B; second incision, A to C.

was then extended ventro-caudally by starting again at point A and proceeding to point C just anterior to the anal opening. To remove the testis or ovary, the mesorchium or mesovarium at the anterior end of the gonad was torn with a pair of forceps and one gonad at a time was lifted ventrally and caudally and laid over the tail of the animal. When both gonads were thus reflected, the gonaducts, which are very short, were freed from the surrounding connective tissue and were then transected. Approximately four individual stitches of No. 2 surgical silk were used to close the incision. These are indicated on figure 1 by the small dots.

As soon as the operations were completed, the fish were placed in individual aquaria containing approximately 5 liters of *Tilapia*-conditioned water in which 30 grams of Louisiana rock salt had been dissolved (0.6 per cent solution). The fish remained in this salt solution until the wounds were completely healed. In previous operations it was found that although fungi grew profusely in the open wounds, this growth could be controlled completely

by placing the fish in a salt solution. *Tilapia macrocephala* is a brackish-water form, and it can be shifted suddenly from fresh water to sea water and vice versa without any apparent deleterious effects. No other aseptic or antiseptic precautions were indicated. The silk sutures, if still present, were removed after a week or two. The operates were judged ready for the experiment when their wounds were completely healed and they had regained their pre-operative weights.

EXPERIMENTAL PROCEDURES

Young adults were paired at random and placed in 54-liter tanks for preliminary spawnings. On the day they spawned, the female was removed from the preliminary tank and, after having been weighed, was placed in one of the experimental situations described above. The stimulus fish was then placed on the opposite side of the glass partition. Although as a rule only one or two weeks were needed to set up a group of four pairs plus the single isolated female, altogether five months elapsed between the establishment of the first and the fifteenth groups.

All experimental tanks were maintained for one year from the date of the preliminary spawning, during which time they were inspected twice daily (except Sundays) for evidence of spawnings as indicated by the presence of eggs. These observations were made between 9 A.M. and 11 A.M., and again between 3 P.M. and 5 P.M. Also, a notation was made of the location of the nests when they could be identified definitely. Any nests built by the stimulus fish on the opposite side of the partitions were also recorded.

Two deaths occurred during the early stages of the experiment. In both cases new pairs were established and these were carried for an entire year in lieu of the original pairs.

After each pair (or individually isolated female) had been observed for one year, the coloration of the operculum was recorded (see p. 12), the fish were weighed and measured (standard lengths), and were then killed with an overdose of urethane. Then the genital tube lengths were measured, and necropsies were performed. For the castrated individuals, particular attention was paid to the possibility of regenerating gonadal tissue. In the intact individuals the macroscopic condition of the gonads was recorded. In all cases the urogenital tracts were dissected out and fixed in Bouin's solution. In nine cases where gonadal regen-

eration was suspected or where the existing gonads appeared atypical, histological sections were made and stained with hematoxylin and eosin, or Masson's trichrome, or both.

RESULTS

The number of spawnings that occurred in each group together with the mean for each experimental situation is listed in table 1. The high mean spawning frequency shown in this table for females on the opposite side of the glass partition from other intact females indicates that a female is equally efficacious as a visual stimulus to another female and possibly even slightly better than a male. The data show, moreover, that a castrated male is as good a stimulus to spawning as an intact male but a castrated female may perhaps be somewhat less effective. (Since the mean differences here approached somewhat the borderline of statistical significance, further statistical tests were used, as described below.) As in the previous experiment (Aronson, 1945), several of the completely isolated females spawned, but the mean spawning frequency was much less than in any of the other cases. In the second experimental situation, where both the experimental and the stimulus fish were intact females, we have in effect two stimulus fish and two experimental fish. These have been designated in table 1 as B and B₁. The B₁ fish, originally designated as experimental, all started the experiment on the day of the preliminary spawning, while the original stimulus fish B were chosen at random from a stock tank and were not tested prior to the experiment. The data in table 1 indicate only a very slight and not statistically significant increase in the mean number of spawnings for the selected experimental females as compared with the randomly chosen stimulus females.

Although the entire experiment was carried out in one room of the greenhouse, the conditions were not so uniform as might be desired. There were temperature differences in the tanks due to distance from radiators; there were differences in the light reaching various tanks and in shadow effects due to differences in the placement of tanks. There were differences in unavoidable transient disturbances according to the nearness of tanks to doors, aisles, etc. To minimize the effect of these uncontrolled factors, all of the tanks of a given group were placed as close as possible to one another. This procedure was considered as justifying the use of the method of individual comparisons described by Snedecor

TABLE 1
FREQUENCY OF SPAWNING UNDER VARYING EXPERIMENTAL CONDITIONS

Experimental Conditions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total No. of Spawnings	Mean and Standard Error (σ_M)	Standard Deviation	Range
Glass Partition Separating:—																			
A Intact male from intact female (control)	6	5	8	9	5	7	4	10	11	11	7	9	9	2	11	114	7.60 \pm .72	2.77	2-11
B Intact female from intact female	4	3	14	9	8	6	13	11	7	11	13	10	1	7	5	122	8.13 \pm 1.00	3.87	1-14
B ₁ Intact female from intact female	5	4	18	7	6	10	13	16	8	8	1	6	9	9	13	133	8.90 \pm 1.18	4.56	1-18
C Castrated male from intact female	5	8	11	9	9	9	9	7	3	15	—	13	4	12	9	123	8.79 \pm .90	3.36	3-15
D Castrated female from intact female	8	0	5	1	—	4	13	2	3	7	8	7	8	2	16	84	6.00 \pm 1.22	4.56	0-16
E Completely isolated female (low-limit control)	0	0	1	1	1	0	0	8	1	4	0	0	3	0	2	22	1.47 \pm .51	2.19	0-8
Total	28	20	57	36	28	36	60	47	36	52	29	48	31	34	56	598			

(1946, p. 43). Accordingly, the difference in the number of spawnings between each pair of experimental situations in each of the 15 groups was calculated. From these figures, the mean differences were determined, as well as the standard errors of these means, from which t and P could readily be calculated. The results of these comparisons are shown in table 2. The upper figure in each comparison is the mean difference, and the lower figure in parentheses is the P value. Here also the lesser spawning frequency for the fish paired with castrated females as compared to the spawning frequency of the control females is somewhat beyond the limits generally recognized as statistically significant. In all the other comparisons, the statistical significance or lack of statistical significance is clear cut. The mean differences were also calculated from table 1 and were found to be very close to the means shown in table 2. Hence these calculations do not reflect the involvement of differences in spawning frequency due to the location of tanks.

TABLE 2

COMPARISON OF MEAN SPAWNING FREQUENCIES OF FEMALES WHEN IN SIGHT OF VARIOUS STIMULUS FISH^a

Stimulus Fish	Intact Male A	Intact Female B	Intact Female B ₁	Castrated Male C	Castrated Female D	Absent E
Intact male A						
Intact female B	+ .5 (> .20) ^b					
Intact female B ₁	+1.3 (> .20)	+ .7 (> .20)				
Castrated male C	+1.1 (> .20)	+1.0 (> .20)	— .6 (> .20)			
Castrated female D	—1.9 (.18)	—2.1 (.19)	—3.1 (.08)	—2.9 (.08)		
Absent E	—6.6 (< .01)	—6.7 (< .01)	—7.4 (< .01)	—7.2 (< .01)	—4.4 (< .01)	

^a Comparisons calculated by pairing individuals in groups. (See text.)

^b Numbers in parentheses are P values.

The control situation of the present experiment is essentially the same as the third experimental condition in a previously reported experiment (Aronson, 1945, table 3), but in that test the tanks were located in two different rooms and hence the data for

the two sets were analyzed separately. From these data the mean number of spawnings for the third experimental condition for the two rooms was found to be 7.6 spawnings per year, which is exactly the figure for the control condition of the present experiment. In the earlier experiment, vision was demonstrated to be the dominant exteroceptive sexual stimulus, but the possibility that chemical or vibratory stimuli might have secondary influences also was noted. This possibility is again favored by the fact that in the present experiment (conducted in room B), with the stimulus fish all separated from the experimentals by solid glass partitions, the females never in any condition reached the high mean spawning frequency of the first two experimental situations in room B of the earlier experiment in which permeable partitions (or lack of partitions) admitted the effectiveness of vibratory and chemical stimuli.

TABLE 3
OCCURRENCE AND LOCATION OF NESTS

Nests built by:	Total No. of Spawn- ings	Spawnings in Which Nests Were Seen		Nests Near Partition ^a	
		No.	Per Cent of Total No. of Spawnings	No.	Per Cent of Total No. of Nests Seen
Intact male	114	19	16.7	17	89.5
Castrated male	123	25	20.3	20	80.0
Castrated female	84	9	10.7	9	100.0
Intact isolated female	22	22	100.0	7 ^b	31.8 ^b
Intact female opposite partition from:					
Intact male	114	109	95.6	104	95.4
Intact female B	233	123	52.8	117	95.1
Intact female B ₁	233	134	57.5	121	90.3
Castrated male	123	116	94.3	106	88.4
Castrated female	84	79	94.0	73	92.4

^a Edge of nest within 5 cm. of partition.

^b Within 5 cm. of either right or left wall.

Additional light is shed upon this problem by an analysis of the intervals between successive spawnings. As in the previous experiment, 27 days was selected as the arbitrary division between short and long cycles. In figure 2, it is seen that situations A, B, C, and to a lesser extent D, closely approximate conditions C and D of the earlier experiment (Aronson, 1945, fig. 2). None of the

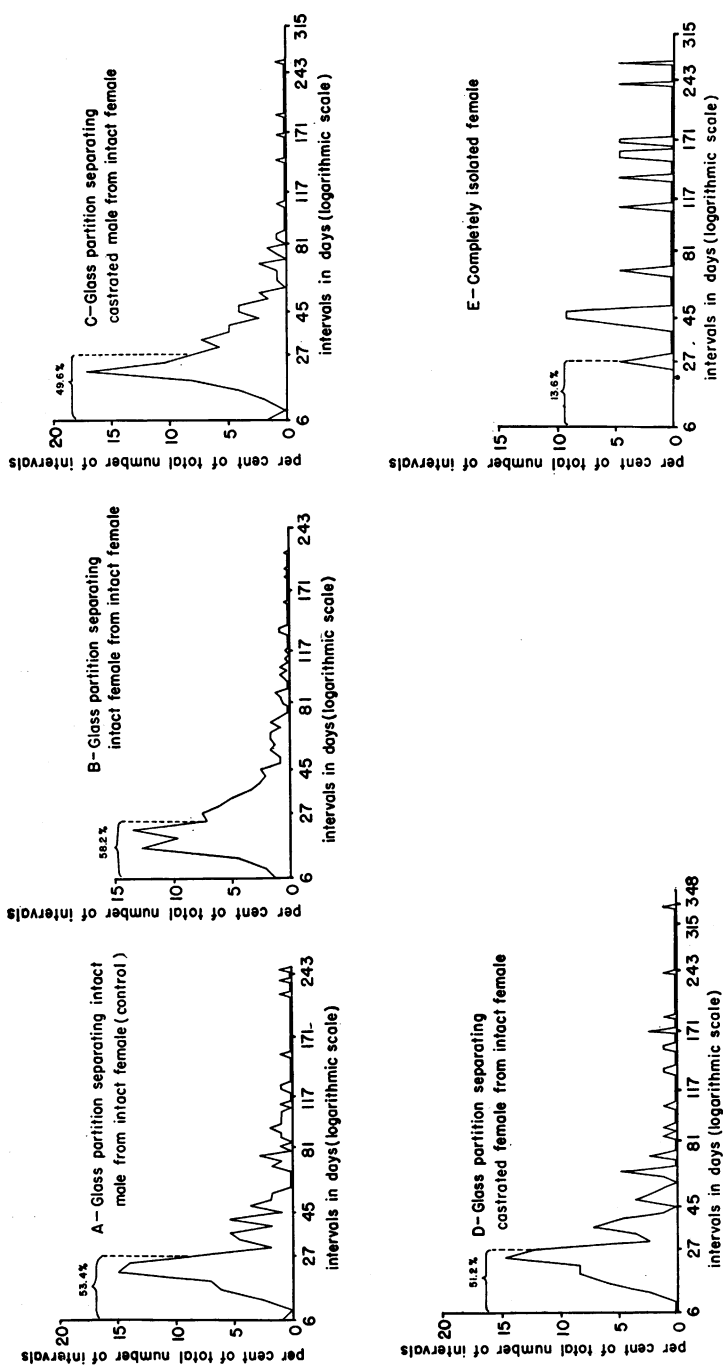


FIG. 2. Graphs illustrating distribution of interspawning intervals in the control and four experimental situations. The intervals in days (abscissa) are plotted on a logarithmic scale. The broken line represents the arbitrary division into short and long cycles, and the percentage of short cycles is indicated in each case.

present experimental situations yielded the high percentage of short cycles seen in conditions A and B of the 1945 experiment. Graph E of figure 2 confirms our previous findings that there is a very marked reduction of the short cycles in completely isolated females, and examination also shows that they were scattered pretty much at random throughout the year. The fact that there was no indication of any superior frequency of spawnings during the early months of the experiment thus supports our previous conclusion that the stimulative effects that the females may have received from other fish prior to the experiment do not carry over into the time when these fish are placed in isolation.

At the time spawnings were recorded, any nests constructed by the fish were identified wherever possible, and the distance from the edge of the nest to the glass partition was measured. These data are summarized in table 3. A study of the behavior of normal *Tilapia* pairs indicated that prior to about 72 per cent of the spawnings, the males did some nest building (Aronson, 1949). In some cases the amount of this nest-building activity was very slight, but in other instances considerable nest building was observed. It is not surprising therefore that, in the present experiment, nests built by the intact males were identified in 16.7 per cent of the spawnings. These undoubtedly represent the cases where the males did sufficient nest building to construct a recognizable nest, whereas any nest building done by the other intact males was evidently insufficient to be identified. Nests built by castrated males were identified in about the same number of cases as the intact males. Nests built by the intact females were identified in a very high percentage of the spawnings when the females were opposite intact males or castrated males or females. In the situation where intact females were separated by glass partitions from other intact females, nests were identifiable in only a little more than 50 per cent of the cases. The reason for this marked decline is not obvious at present but may be clarified by further behavioral observations. Castrated females built identifiable nests in 10.7 per cent of the spawnings, while the intact isolated females built identifiable nests every time they spawned. It is clear from these data that castration did not markedly interfere with the nest-building activity of males, but, in the female, loss of the ovaries reduced this activity to the level of intact and castrated males.

In the 1945 experiment previously referred to, it was observed

that the nests built by the stimulus fish as well as by the test females were always close to the transparent barriers. The data of the present experiment confirm and expand that observation. In all the experimental situations except that of the completely isolated female, a high percentage of the nests of both the stimulus fish as well as the test females was located within 5 cm. of the partition (table 3). As for nests of the completely isolated females, only 31.8 per cent were located within 5 cm. of either the right or left wall of the tank. This would be about the number expected if the nests were distributed at random throughout the tank.

CASTRATION EFFECTS

External morphological changes occurring as a result of castration have not yet been reported for any cichlid fish. It was considered appropriate therefore to study and report such changes, especially since it would be important to consider these morphological differences in relation to the stimulus values of the castrated fish. Because preliminary observations indicated the possibility of castration-induced changes in (1) the genital tube, (2) the coloration of the operculum, and (3) in general growth, when the experiment was terminated, measurements were made of these three characters. The findings are described briefly below.

EFFECT ON GENITAL TUBE

The genital tube in *Tilapia* is a small, flaccid, semitransparent papilla situated just caudad to the anus. The genital ducts extend through this papilla and open at its tip. The mesonephric ducts also extend into the papilla and unite with the genital ducts very close to the tip of the genital tube. The tip is slightly notched, thus forming two small lips around the urogenital opening. The papillae are quite small in immature individuals and grow noticeably as the animal matures. Just before a spawning, both male and female rub their genital tubes over the bottom of the nest. At this time the tubes become further enlarged and turgid, a change which indicates that oviposition time is approaching.

With a pair of small dividers, measurements were made from the base to the tip of the genital tube. Care was taken to stretch each individual tube by approximately the same amount. The

results of these measurements are seen in table 4. The female tube proves to be significantly longer than that of the male. Moreover, the female tube appears somewhat stouter and also less pointed than that of the male, so that with experience one can sex the fish on the basis of this character. Castrations reduced the size of both the male and the female tubes significantly, but it is interesting to note that among the castrates the length of the tubes of the castrated male and female did not differ. Among the 30 operated individuals used in this experiment, postmortem examination revealed that actually only one male had been incompletely castrated and that in one female ovarian tissue had regenerated. The genital tube lengths of both of these individuals measured 2.5 mm., which is well within the range of normal variation for intact males and females. Noble and Curtis (1939) found that in the jewel fish, *Hemichromis bimaculatus*, the tube of the male is also somewhat smaller than that of the female, and the fish can be sexed by means of the slight differences in size and shape.

TABLE 4
COMPARISON OF GENITAL TUBE LENGTHS OF INTACT AND CASTRATED *Tilapia*

	Intact Male	Intact Female	Castrated Male	Castrated Female
No. of individuals	14	84	12	13
Mean length in mm. ($\pm \sigma_M$)	2.2 \pm .07	3.0 \pm .06	1.2 \pm .09	1.0 \pm .09
Standard deviation in mm.	.25	.57	.31	.33
P values:				
Intact female	< .01			
Castrated male	< .01	< .01		
Castrated female	< .01	< .01	> .10	

COLORATION OF OPERCULUM

The operculum of the immature *Tilapia* is silvery. As the males mature, the silvery appearance is replaced by a bright golden yellow surface. As the females mature, a red spot appears in the center of the operculum. Subsequent examination of the female operculum (Aronson and Holz-Tucker, MS, in preparation) has revealed that this red spot in the female is in effect a

semitransparent window in the center of the operculum through which the red of the gills shows. Hence the maturation of the female evidently induces a resorption of the silvery guanine crystals in the central region of the operculum.

At the close of the experiment, three persons were asked to record the opercular coloration of each of the fish under one of the following seven categories: (1) bright yellow, (2) dull yellow, (3) faint yellow, (4) no yellow or red, (5) faint red, (6) dull red, (7) bright red. In most cases the three observers were in close agreement. In several cases they differed by not more than one step. However, in a few cases, as noted below, the differences were greater. In the summary of these observations (table 5) the average for the three observers was used. It is seen that in most cases the opercula of the intact males were bright yellow. In a few cases the color was less intense. Two possible explanations may account for the red reported in the two cases listed in table 5. One is that the yellow layer was quite thin and that some red from the gill showed through.

TABLE 5

SUMMARY OF OBSERVATIONS ON OPERCULAR COLORATION

Intact males	12 bright yellow (in one case two observers saw some red) 3 dull yellow (in one case one observer saw some red)
Intact females	32 bright red 48 dull red 9 faint red
Castrated males	5 no yellow or red 8 faint yellow 1 dull yellow (one observer called this bright yellow)
Castrated females	3 no yellow or red 9 faint yellow 1 faint red

It is more likely, however, that some of the melanophores in the operculum were expanded and presented a dark spot or spots which the observers mistook for red. The central portions of the opercula of the female were either bright red or dull red in most cases. The differences apparently represent the degree of transparency of the window. In nine females the red coloration could be seen only faintly. These cases will be considered in further

detail below. With one exception, the opercula of the castrated individuals were either faint yellow or entirely silvery, with no yellow or red at all. In the exceptional case with the dull to bright yellow operculum, the urogenital system was sectioned serially, but no trace of gonadal tissue was revealed. It is possible that a small disconnected piece of testis, perhaps from the anterior end, may have passed unnoticed during the autopsy, to be discarded with the viscera, or this male may have had an unusual extra-gonadal source of testis hormone.

Since the red spots in the intact female appear to be related to the presence of the ovary, the data for the nine females with faint red opercula were examined in detail. These data are summarized in table 6. It can be noted that these special females were found in all the experimental situations except the controls. Moreover, the number of spawnings varied from zero to 13, indicating that the opercular coloration in these cases was not related to the frequency of ovulation or spawning. However, the data on oviposition were gathered throughout an entire year, whereas the opercular coloration was measured on the last day of the experiment. Hence the possibility of a change in ovarian activity must be considered. In the last column of table 6, where the number of days between the last spawning and the termination of the experiment is indicated, it can be seen that several of the females with the poorly colored opercula spawned quite close to the day on which the coloration measurements were taken. Column 5 of table 6 demonstrates that the genital tube lengths of these last-mentioned females were up to normal, a fact that may be taken to indicate that the hormonal activity of the ovaries of these fish had not been impaired. Because all the females selected at the beginning of the experiment had prominently red opercula, the loss of coloration must have occurred during the course of the experiment. Hence this loss appears to be unrelated to ovarian disfunction, despite the fact that the female castrates also lost the red spot. These observations also suggest that the appearance of the genital tube is a better indicator of reproductive activity in the female than the opercular coloration and should be used for the purposes of selecting breeding fish. Subsequent investigation (Aronson and Holz-Tucker, MS, in preparation) has shown that slight fluctuation in opercular coloration is a frequent occurrence in females, but correlation with the ovarian cycle has not yet been established.

TABLE 6

SPAWNING FREQUENCY OF NINE INTACT FEMALES HAVING FAINT RED OPERCULA

Female No.	Group	Experimental Situation	No. of Spawnings	Genital Tube, Length in Mm.	Interval between Last Spawning and End of Experiment
75	XI	B	13	2.7	10 days
92	XIII	B	1	2.2	10 months, 21 days
15	II	B ₁	4	3.2	4 months, 10 days
93	XIII	B ₁	9	2.8	0 days
18	II	C	8	2.8	1 month, 14 days
54	VII	D	13	3.0	3 days
17	II	E	0	3.0	—
22	III	E	1	3.0	4 months, 5 days
77	XI	E	0	2.7	—

EFFECT ON GROWTH

The effects of castration on growth are summarized in table 7. The intact males and castrated males gained approximately the same amount, the mean difference not being significant. The castrated females gained an equivalent amount, but the intact females gained very much less. The latter were slightly smaller to begin with, but since unpublished data indicate that the growth rate is linear during this stage, the small differential at the beginning of the experiment cannot account for the large difference between the weight gains of the intact and castrated females. It is well known that ovarian hormones may influence general metabolism and growth rates in vertebrates, and Svärdson (1943) has verified this for the guppy, *Lebistes reticulatus*. Hence we were inclined at first to attribute the slower growth rate of the intact females to the effect of their ovarian hormones on general metabolism. However, since the other three categories all gained approximately the same amount and since these weight gains were also reflected in comparable length gains, we looked for other possible explanations. The large number and size of the eggs immediately came to mind, and this possibility was analyzed as follows: The mean weight of the intact females during the entire year was approximately 13.5 grams. According to a previously determined regression line (Aronson, 1949, fig. 16) such females laid about 64 eggs per spawning. The 88 females measured in this experiment spawned 598 times and therefore laid

about 38,272 eggs. The mean weight of a sample of 43 eggs laid by a 20-gram female was 15 mg. The mean weight of a sample of 37 eggs laid by an 8-gram female weighed 11 mg. (unpublished data). Therefore our average female probably laid eggs weighing around 13 mg. Multiplying these factors, we find that our 88 intact females laid 497 grams of eggs, or a mean of 5.6 grams. If this figure is added to the mean gain for the intact females, we arrive at a total of 15.2 grams.

TABLE 7
SUMMARY OF WEIGHTS AND LENGTHS

	No. of Fish	Mean Starting Weight $\pm \sigma_M$ (gm.)	Mean Gain $\pm \sigma_M$ (gm.)	Com- parison of Gains (P Values)	Mean Standard Length (cm.)
Intact males	15	8.9 \pm .73	18.4 \pm 1.0	> .10	9.7
Castrated males	14	9.1 \pm .98	20.4 \pm .99		9.8
Intact females	88	8.0 \pm .28	9.6 \pm .44	< .01	8.4
Castrated females	13	10.2 \pm .87	18.5 \pm .98		9.4

From these somewhat crude approximations, it is seen that most of the growth differential between the intact and castrated females can be accounted for reasonably by the loss of eggs. That is, we may say that in the castrated individuals most of the food was applied towards body growth, while in intact individuals a good portion of the available food was spent manufacturing eggs. It should be borne in mind that during this experiment the fish were fed entirely with dry food which cannot readily be offered *ad libitum*. The feeding method consisted of introducing an amount of food estimated to be just about what the fish will rapidly consume. Although this method is entirely satisfactory for raising fish, it may be a source of considerable error, particularly in problems of growth. It is possible that the intact females would have consumed more food if it had been offered. This difficulty could be overcome by having some live food available at all times. Fish are unusual among the vertebrates in that in many species growth continues long after reproductive maturity, and their size may increase manyfold. In certain teleost species, females are often somewhat smaller than males, and it could very well be that this is not caused directly by differential growth rates, but rather indirectly by a diversion of part of the available food of the females to the production of eggs.

DESCRIPTION OF INCOMPLETE CASTRATIONS

In one operated male a small piece of testicular tissue about 3 mm. in length was found at the posterior end of the peritoneal cavity attached to the genital duct. The operculum of this incomplete castrate was recorded as bright yellow by all three observers. The genital tube length measured 2.5 mm., which is slightly above the average for intact males. It would appear that even a small piece of testicular tissue is sufficient to maintain a normal genital tube and opercular coloration. Normal testes measure about 2 cm. in length each. The regenerated testis was approximately 3 mm. long. Since the widths were comparable, it is estimated that 7.5 per cent of the normal amount of testicular tissue was sufficient in this case to maintain normal sex characters. These figures compare favorably with those of St. Amant (1941, quoted by Turner, 1942), who found that a piece of testis one-forty-fifth of the normal volume was capable of producing enough hormone for complete development of the gonopodium of *Gambusia*. Later Turner (1947) reported that "a regenerated testis" of *Gambusia* "containing only four per cent of the volume of normal mature testis is sufficient to cause rapid renewal of the accelerated growth rate and to carry gonopodial development to completion."

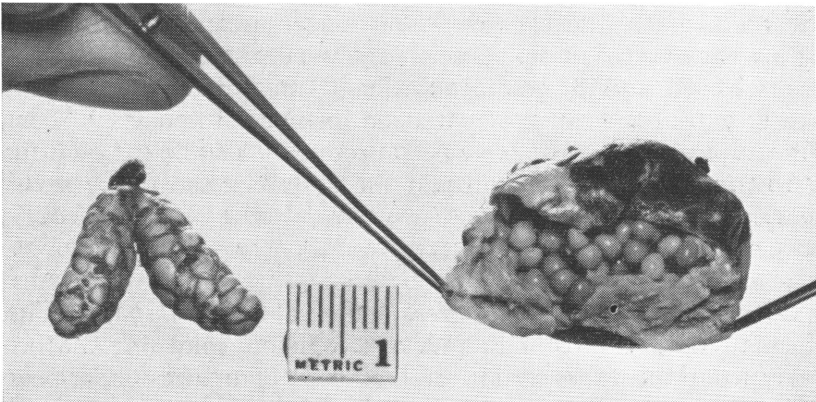


FIG. 3. Right: Regenerated cystic ovary cut open to show ovulated (loose) eggs and thick opaque ovarian wall. Left: Normal mature ovary. Note that the ovarian wall is thin and transparent.

After the experiment had been in progress for about six months, it was very evident that operated female no. 13 was developing

an intra-peritoneal growth. Necropsy at the termination of the experiment revealed a large, slightly ovoid mass 2.5 cm. and 2.1 cm. in diameter adhering by connective tissue strands to the dorsal wall of the peritoneal cavity (fig. 3). This mass had a thick, tough wall, much of which contained heavy black pigment similar to that found on the peritoneal lining. A slit was made in this wall, which revealed that it was entirely filled with large eggs, many of which were loose and not in follicles. Some of the eggs appeared to be in different stages of disintegration. Along the inside of the wall numerous follicles containing small eggs could be identified readily. It is believed that this mass was a regenerating ovary disconnected from the oviduct and that the eggs were being ovulated into the sac without any possibility of escape. In addition to this large mass the wall of the peritoneum contained innumerable small ovoid bodies of varying sizes, most of which were also heavily pigmented. Sections of these masses stained with hematoxylin and eosin and also Masson's trichrome indicated that the walls of these masses were composed of connective tissue, and in the center material that resembled degenerating yolk was found. It is possible that in the early stages of the ovarian regeneration the wall was quite thin or perhaps even incomplete and that the eggs at first were being ovulated into the ovarian cavity where they became encysted. Later, as the wall became thicker, the eggs could no longer escape and remained within the ovarian mass. The operculum of this fish was reported as bright red, and the genital tube length measured 2.5 mm. which is well within the range of variation for normal females. Hence this atypical mass must have been producing a sufficient quantity of ovarian hormone to maintain the described secondary sexual characters.

DISCUSSION

Observations now in progress indicate that *Tilapia* females normally ovulate within an hour or two before spawning. Moreover, from the examination of hundreds of ovaries, it appears that females rarely, if ever, retain batches of ovulated eggs for very long. It is likely then that in the present and preceding experiments we are dealing primarily with ovulation frequencies and only secondarily with oviposition. With the foregoing assumption in mind, we can hypothesize that ovulation in *Tilapia* is influenced by a multiplicity of factors suggested by these studies.

The observation that some completely isolated females continued to spawn could possibly be attributed to stimuli emanating from small general changes in the environment which could not be eliminated under the conditions of our experiment. For example, disturbances unavoidable in the daily inspections, reflections in the glass, shadows, and light changes coming from the top of the tanks may all have had some slight stimulus values. It is more likely, however, that we are dealing here with internal physiological activities, particularly hormonal levels and metabolic rates, which are relatively independent of exteroceptive influences. Moreover, it is clear that this is a threshold phenomenon. About half of the completely isolated females never reached this threshold for ovulation, while a few not only surpassed the threshold but maintained a spawning frequency in line with our control pairs. Thus the first factor influencing ovulation may be considered an internal physiological one, dependent perhaps on the general metabolic state of the individual.

Because the four different stimulus fish used in the present experiment raised the ovulation frequency of the test females considerably above that of the completely isolated fish, the second factor may be described as the visual stimulus of the partner. The possibility suggests itself that fish of different species, crude models of fish either stationary or moving around in the tank, or perhaps just a shadow may be at least partially effective in stimulating females to ovulate. Future experiments may determine whether or not this second factor can be subdivided in this way on the basis of the complexity of the stimulus pattern. In this connection, it is interesting to note that in a considerable variety of stimulus-response experiments performed on a number of very different species of fish (Lissman, 1932; Noble, 1934; Peters, 1937; Seitz, 1940; and Tinbergen, 1948) relatively simple models adequately elicited specific behavioral responses. In the case of the guppy, even the projected shadow of a different species of fish was sufficient to elicit a specific courtship response (Breder and Coates, 1935). Unfortunately the above experiments were not sufficiently quantified to indicate the relative efficiency of these artifacts.

It might not be fair to compare too precisely the data of the earlier experiment with those of the present one, since the conditions were not altogether comparable. On the other hand, these changes (better diet, cleaner tanks) made for better maintenance

and health of the fish, reflected in a lower total of deaths during the present experiment. There is little likelihood that these altered conditions could have lowered the spawning rate to any extent. Yet none of the spawning frequencies this time reached the high levels reported for situations A and B of the previous experiment (in which male and female were separated by the grating, or the male and female were together in the same aquarium). These differences which have been found statistically significant may be interpreted as either chemical, vibratory, or

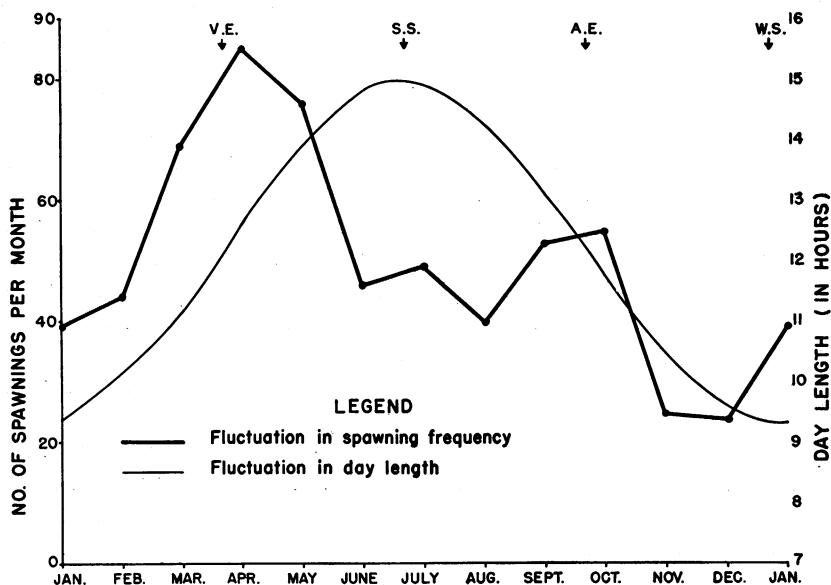


FIG. 4. Graph showing seasonal distribution of spawnings when the number of egg layings per month for all the experimental situations were added together. The thin line representing fluctuation in day length was plotted from the sunrise and sunset tables of the American Nautical Almanac, 1949. *Abbreviations:* A.E., autumnal equinox; S.S., summer solstice; V.E., vernal equinox; W.S., winter solstice.

possibly a combination of both, and represent the third in a series of factors affecting ovulation.

Light and temperature have not been investigated to any considerable extent, but general laboratory observations indicate that when the water temperature drops much below 20° C. the fish fail to spawn. Similarly it appears that spawnings increase in our laboratory greenhouse during a series of bright sunny days

and drop noticeably during extended periods of dull weather. Light and temperature are undoubtedly important factors influencing ovulation. Moreover, a pronounced seasonal fluctuation in spawning frequency was demonstrated by plotting the total number of spawnings for all the experimental situations in a monthly distribution graph (fig. 4). The major peak in March coincides with the vernal equinox; a lesser peak in October roughly coincides with the autumnal equinox, while the two troughs occur at the times of the solstices.¹ The graph suggests that the fish were being influenced not so much by the total amount of daylight as by periods of greatest daily change, wherein rapidly increasing day length was the strongest stimulus to ovulation and oviposition while rapidly decreasing light was a lesser stimulus. On the other hand, the phenomenon can also be explained by postulating the existence of a minimal threshold of light necessary for ovulation. We may say that with the increased day length in the spring the threshold for most females is surpassed, and a peak of spawning activity results. However, metabolic exhaustion, or perhaps a state of refractoriness of the anterior pituitary gland or gonads as suggested by Bissonnette (1937) and Miller (1948), prevents the continuance of this high reproductive rate, and the number of spawnings decreases despite the long hours of daylight. Recovery during the summer permits a second wave of spawnings while day lengths are still sufficiently long, but as the light again drops below the threshold for most of the fish, the spawnings drop to the lowest frequency for the year, coinciding with the winter solstice.

A survey of the literature indicates that in vertebrates the majority of seasonal breeders reproduce either in the spring or fall, corresponding roughly to the two peaks exhibited by *Tilapia*. However, some spring breeders exhibit a limited amount of reproductive activity in the fall. Thus Noble (1931) referring to the Salientia (frogs and toads) writes, "In the fall with the ripening of the gonads, some northern and many southern frogs begin to call persistently. There are several records of species which normally breed in the spring, having laid in the fall." Noble (1937) states that "Although most snakes living in the north temperate region breed in the spring, a seasonal oestrus

¹ A chi-square test gave a *P* value of $< .01$, indicating that the fluctuation in spawning frequency was statistically significant.

apparently occurs normally in the fall in the case of many species. September matings have been recorded for *Natrix natrix*, . . . *N. tessellata* . . . as well as in *Thamnophis radix* . . .” The British race of the starling, *Sturnus vulgaris*, which normally breeds in the spring, exhibits a marked period of gonad recrudescence and sexual activity in the fall, and the same appears to be true for several other species of British birds (Bullough, 1942).

In wild rodents, which have continually recurring estrous cycles, breeding usually ceases during the winter months. *Mus musculus*, the house mouse, appears to be exceptional in that in the wild it breeds throughout the year at a very even rate (Laurie, 1946). In this article a graph of the seasonal distribution of pregnancy of a group of fecund mice living in corn ricks shows two peaks, one in March and the other in September. These correspond roughly to the two peaks found in *Tilapia*. Although these mice frequently come out of the ricks and hence are exposed to the possible influences of seasonal variations in day length, Laurie minimizes the importance of his finding since a chi-square test which he ran gave a *P* value which was not significant.

Tilapia seem to spawn equally as well in salt and fresh water, and small fluctuations in pH do not seem to affect ovulation to any degree. However, these factors have not been examined quantitatively.

Thus far in our experiments we have been dealing with individual fish as stimulus objects. Whether or not numbers would have a greater effect has not been ascertained. When several of these fish were placed in an outdoor garden pool several summers ago, it was noticed that they schooled to a considerable extent¹; hence an additive effect is possible and should be investigated.

Little evidence has been published concerning the natural habitat of these fishes, but a very closely related species, *Tilapia heudeloti*, which may be synonymous with *Tilapia macrocephala* (Boulenger, 1915), has been reported as living in the river delta lagoons along the Gold Coast and related areas of West Africa. At times these lagoons are entirely cut off from the sea and may be in communication with rivers; at other times they are flooded by the sea. In such situations the salinity varies erratically

¹ However, Dr. C. M. Breder, Jr., to whom the author is indebted for this observation, notes that the continuous low temperature of this pond (averaging about 60° F. between June 1 and September 1) might have been responsible for the schooling behavior.

(Irvine, 1947). While the specific conditions under which these *Tilapia* spawn in the wild are not known, it is very conceivable that a series of exteroceptive factors hastening or inhibiting spawning could be of considerable adaptive significance in a rapidly fluctuating environment like that of *Tilapia* in nature.

While the data of the present experiment failed to establish statistical significance in the decreased spawning frequency of females separated by transparent partitions from castrated females, as compared to the control females, this drop still cannot be eliminated as a possible factor. It is worth while therefore to consider how the stimulus values of castrated females could differ from those of the other stimulus fish used in this experiment. Morphological factors are not likely to be important, since in the two striking differences between males and females, namely, the dimorphic opercular coloration and genital tube size, the castrates of both sexes are alike. Noble and Kumpf (1936), in a preliminary study of sexual behavior in a related cichlid, namely, the jewel fish, *Hemichromis bimaculatus*, report that castration abolishes courtship behavior in the female but not in the male.

Observations on the courtship and pre-spawning activities of castrated *Tilapia* will undoubtedly throw further light on this question, although we already can be certain from the appearance of nests that castration does not abolish nest building in either sex.

The results of the present and previous experiments indicate that the sight of another fish is the major effective stimulus for ovulation and oviposition. Sexual adornments and complex courtship patterns exert at the most minor influences. Form may be important but is as yet untested. Thus we can postulate that the basic mechanism is a part of the well-known optic-hypothalamic-anterior pituitary-gonad-sex behavior system.

On the other hand it is possible that the stimulus effect found in this investigation may be much more general and diffuse. Allee (1931, 1934) has emphasized that many physiological processes are seriously affected by the number of individuals present in a limited space. Shlaifer (1938) found that in a given volume of water an isolated goldfish consumes more oxygen and has a higher rate of locomotor activity than does each fish in a group of two. Further experiments indicated that this effect was lost when the animals were blinded or were placed in the dark. Shlaifer (1939) concludes that vision is a major sense involved in this aspect of mass physiology, and it is more likely that this

effect is due to the perception of form rather than color or movement of the fishes comprising the group. In a similar manner it can be hypothesized that the appearance and perhaps the behavior of a second fish influence the locomotor activity and general metabolic processes of the female *Tilapia*, and the reproductive processes are thus affected only indirectly as a result of this more general stimulation.

SUMMARY AND CONCLUSIONS

The visual stimulus responsible for the high oviposition frequency of female *Tilapia* was analyzed by utilizing experimental aquaria in which females were separated from a variety of stimulus fish by transparent glass partitions. It was found that the mean spawning frequency of intact females on the opposite side of glass partitions from other intact females was slightly higher than the means for the controls (i.e., females opposite intact males). Females opposite castrated males spawned on the average as often as the controls. Females opposite castrated females spawned much less than the controls, but the statistical significance of this reduced rate could not be demonstrated because of high variability. All the intact females mentioned above spawned on the average considerably more frequently than a group of completely isolated low-limit control females.

Certain morphological changes occurring as a result of castration were also studied. The genital tubes of both the male and the female were reduced in size after gonadectomy. Following removal of the testes, the bright yellow surface of the mature male operculum assumed a silvery appearance characteristic of immature *Tilapia*. Ovariectomy caused the bright red spot, which normally appears in the center of the mature female operculum, to acquire a similar silvery appearance. However, some females with apparently normally functioning ovaries did not maintain the typical red spot, and hence this character is not a good indicator of ovarian function. Weight gains for the intact males, castrated males, and castrated females were approximately the same during the course of the experiment, but the intact females gained considerably less weight. Calculations indicated that this differential roughly approximated the mass of eggs laid by the females during the experimental year.

The study suggests that the factors affecting ovulation in *Tilapia* can be classified as (1) internal or physiological; (2)

exteroceptive stimulation by a second fish in which vision plays a predominant role, and chemical or vibratory factors may be of lesser importance; (3) environmental, especially light and temperature.

A seasonal fluctuation in ovulation frequency, with a major peak in the spring and a minor peak in the fall, is correlated with fluctuations in day length.

The striking differences in opercular coloration among males, females, and castrates do not appear to affect the stimulus values of the fish, but possible differences in courtship behavior, especially between castrated females and intact individuals, may be important and will be investigated shortly.

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