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INTRODUCTION

A NUMBER OF GROUPS of marine teleosts which inhabit tidal regions present similar complex patterns of territorial and reproductive behavior. Prominent among the tidal zone residents are members of the Gobioidea and Blennioidea. Among the best of the early descriptions of spawning were those of Guitel (1892, 1893, 1895) on several species of European gobies and blennies. Most subsequent reports were based on very few specimens.

The "mapo," *Bathygobius soporator* (Cuvier and Valenciennes), was found to be common in the vicinity of Marine Studios, Marineland, Florida, where a number of animals were collected during the summer months. Spawnings were observed to take place readily in the aquaria at the Marineland Research Laboratory.

This species is known for its possession of several rather unusual features in its structure, embryology, and behavior. Beebe (1931) remarked on the presence of a number of free, silk-like fin rays on the upper margins of the pectoral fins. These structures were alleged to serve as accessory respiratory organs. The remarkably developed color-changing ability of *Bathygobius* has been studied by Breder (1948, 1949) and Tavolga (1950a). The extremely elongate shape of the eggshell in this species is formed, during water-hardening, into a sarcophagus-like container within which the larva fits snugly. The curious shape of these eggs prompted Breder (1943) to investigate the geometric properties of non-spherical fish eggs in general. Tavolga (1950b) described the embryonic development in *Bathygobius*. The ability of these gobies to leap unerringly from tide pool to tide pool was studied by Aronson (1951b) in relation to their topographic memory and learning capacity.

Based on data accumulated at Marineland, a descriptive study was undertaken of the reproductive behavior of this species, and the present report comprises a qualitative description and some notes on the variability of the behavior pattern.

MATERIALS AND METHODS

SOURCE AND CARE OF MATERIAL

MOST OF THE SPECIMENS used in this work were collected from large tide pools near Matanzas Inlet, about 3 miles north of Marine Studios. Others were collected from several points on the inland waterway in the vicinity of Marine Studios.

Individuals used here ranged from 40 mm. to 90 mm. in standard length.

The description of spawning behavior is based on observations of eight males and 17 females. Other behavioral studies involved an additional 12 males and 13 females.

Most of the animals were maintained in 10-gallon aquaria (a few were kept in small 2-gallon tanks), supplied with running sea water. As many as eight animals per 10-gallon tank could be maintained, but fewer were generally used because of the pugnacious behavior within this species.

Minced, fresh shrimp was fed once a day. Food was given at each feeding until the animals no longer displayed interest, and no food was permitted to remain in the tank uneaten.

In line with the secretive habits of this species, shelters were provided in the aquaria, both for spawning and for hiding. The first three spawnings observed took place in small conch shells and oyster shells, but for all subsequent spawnings unglazed flooring tiles were provided. These tiles were a light brown color and measured 4 inches by 4 inches by ½ inch. They proved to be remarkably convenient for this work, since they could be arranged into a shelter by leaning one against the front glass of an aquarium and using another for the floor. The triangular shelter thus formed was readily used by the gobies for hiding and spawning. The tiles combined maneuverability, standardization, and ease of observation, and they could also be removed periodically to check the stage of development of eggs deposited on the surface.

SPAWNINGS OBSERVED

As shown in table 1, a total of 21 spawnings was observed in the laboratory, and two spawnings were observed, in part, in the field. Some of the egg layings involved more than one female, and in other cases single individuals spawned more than once. Three of the males and one of the females bred four times each; one male and two females bred three times each; two males and five females bred two times each; and the remainder each spawned once.

Among the females that oviposited more than once, the interval between spawnings ranged from seven to 16 days, averaging about 10 days. Males could fertilize a batch of eggs shortly after the hatching of a previous brood, i.e., five days after a prior spawning.

SECONDARY SEX CHARACTERISTICS

As in all gobies, the urogenital papillae of the two sexes are easily differentiated. The urogenital papilla of the male is long, conical, and usually heavily peppered with melanophores (pl. 24, figs. 1, 3). The papilla of the female is shorter, truncate, and tipped with two bunches of short, fine, epidermal papillae (pl. 24, figs. 2, 4). The urogenital papilla of the female is only slightly pigmented, if at all. These papillae normally lie flat against the body, pointing caudad from the anus. Pressure upon the abdomen of a female causes the papilla to erect distinctly (pl. 24, fig. 4), whereas this is not true of that of males.

The largest females collected were 65 mm. in standard length. Males were found to reach a length of 90 mm. or more.

In large males, the last rays of the dorsal and anal fins overlap the base of the caudal fin. This is not true of even the largest females. In the latter, when the posterior dorsal rays are appressed caudad, the tips of the last two rays fall from 1.5 to 3.0 mm. short of the base of the caudal fin. In males below 60 mm. in standard length, this distance varies from 3 mm. to zero, i.e., the tips of the fin rays just making contact with the base of the caudal when appressed. In males from 60 mm. to 70 mm., the distance from the tip of the dorsal ray to the base of the caudal varies from 0.5 mm. to an overlap of up to 0.5 mm. In males over 70 mm. in standard length, the extent of overlap may reach 4.5 mm. in some specimens. A similar, correlated overlap of

TABLE 1

LIST OF SPAWNINGS OBSERVED IN *Bathygobius*

Spawning Number	Date Observed	Male	Female
1	July 5	A	1, 2
2	10	B	3
3	15	C	4
4	22	D	5
5	25	C	3
6	25	E	6
7	26	B	4, 7, 8
8	29	D	5
9	30	F	9
10	30	A	10
11	30	G	11
12	Aug. 1	E	6
13	5	B	4, 8, 12
14	6	C	10
15	7	H	13
16	8	D	5, 14, 15
17	9	E	6, 11
18	11	C, B	16
19[a]	12		
20[a]	15		
21	17	D, C	12
22	17	C	4
23	21	H	17

[a] Observed in the field.

the anal fin is present, but was not measured because the tips of the anal fin rays were frequently worn or broken, which made such measurements inaccurate.

Coloration differences between males and females are distinct only during phases of reproductive behavior. These differences are described below.

Bathygobius soporator is usually found in shallow regions with sandy or shell bottoms and in tide pools and is rarely present in mud flats. In the vicinity of Marineland, the bottom is mostly muddy and littered with oyster shells. Occasional, small areas are present with exposed sandy bottoms, and the gobies are found restricted to such areas.

In these sandy areas, gobies are always to be found in or near some shelter. At Matanzas Inlet, oyster shells were plentiful and commonly used as hiding places by the majority of secretive species in that region. A few empty conch shells placed in the tide pools were found to contain large male gobies when examined three days later. In one such shell, a spawning had taken place, and, as judged by the stage of development of the eggs, oviposition occurred within 24 hours after the shell was planted.

In aquaria, any available shelters, including the tank corners, were utilized and fought over.

As is the case with most gobies, blennies, and other territorial teleosts, members of this species are pugnacious with respect to one another, and there is an almost continuous nipping and chasing in a tank with several individuals. A variable "peck" order is formed which appears to be correlated with size. This is particularly evident in homosexual groups, where the largest animals dominate the shelters and do most of the nipping and chasing. The smaller specimens retreat to higher levels, hanging on the tank walls and corners by means of the suction of of their fused pelvic fins, or at times attempting to jump out.

In heterosexual groups, males are distinctly dominant, in terms of holding shelters and nipping, over females of equal size.

The coloration of this species is variable and changes rapidly in response to different environmental and social situations. The fundamental pattern consists of four broad, dark, transverse bands on the body and tail, with less distinct bands across the nape and occiput. These bands become confluent on the sides and irregularly broken towards the venter. The color and intensity of the bands vary from a dense, velvety black through a series of intermediate browns and tans to complete invisibility. The ground color between the bands varies from black in the darkest phases to a light yellowish tan. When completely darkened, the bands become indistinguishable from the ground (pl. 25, fig. 3). In the lighter and intermediate color phases, two of the cross bands remain distinct, whereas the other markings are pale (pl. 25, figs. 1, 2). These patterns were among those most frequently observed both in captivity and in the field. Males frequently possess a darkened chin and throat in connection with the two-bar pattern. Large males, when isolated, generally exhibit a less definite pattern, with the transverse bands not sharply delimited. When dark adapted, i.e., in shelters, the bands are obscured by longitudinally arranged series of small white spots on the sides of the body and tail.

Color changes in this species are rapid and, generally, closely correlated with conditions of incident light and background shade (Tavolga, 1950a). Certain color changes were found to be associated with dominance and aggressive behavior. Aggressors are darker, with the dark bands standing out strongly against a lighter ground color. The retreating animal is usually lighter but responds quickly to background changes, e.g., turning dark upon reaching one of the back corners of the tank. Breder (1949) reported this type of behavior in *Bathygobius*, where the dominant individual displays a background-contrasting color while the subjugated animal exhibits background matching.

Pugnacious behavior among gobies reaches its peak in encounters between mature males of about equal size. They approach each other with slow undulations. Within 10 or 15 seconds, their color becomes extremely dark, sometimes a patternless, velvety black (pl. 25, figs. 3, 4; pl. 26, figs. 1, 2). All the fins are stiffly erected, with a sharply defined light yellow band across the distal ends of the anal and posterior dorsal fin rays.

The two males place themselves next to each other, in a head-to-tail position. Then the body of one quivers, the head is raised,

the throat puffed (pl. 25, fig. 4), and the mouth and opercula are widely gaped (pl. 26, figs. 1, 2). A quick butting or biting movement follows. The two males may go through this behavior alternately or simultaneously. In biting, the fins or opercula are grasped vigorously, often resulting in considerable damage. Occasionally they lock jaws for several seconds. If the antagonists are of nearly the same size, the combat may continue for as long as an hour or more before one retreats. In a small tank, the fighting will continue for several hours, or until one of the animals is killed. In one case, two males separated by a partition of lucite attempted to continue this combative behavior for four days (pl. 26, fig. 2).

Combative behavior was observed between a male and a female only once. The two animals were 60 mm. and 58 mm. in standard length, respectively. The male was the aggressor throughout, and the combat lasted for less than one minute, after which time the female retreated. In heterosexual groups, males generally nip and chase the females but do not, with the above exception, exhibit this intensive color change and attacking behavior.

In three cases, pairs of females were observed to close combat in the above manner. Much of the behavior was identical with that of males, i.e., body quivering, throat puffing, gaping, and biting. The coloration of combating females was not a complete black, and the transverse body bars were always distinct (pl. 26, fig. 3). The yellow banded dorsal and anal fins have not been observed in females. The fighting never lasted for more than 10 minutes at a time. In one case, a pair of females (each about 50 mm. in length) were confined in a small 2-gallon tank. Fighting took place sporadically over a three-day period and then ceased. No serious damage was ever noted in any of these cases. The females illustrated (pl. 26, fig. 3) were separated by a lucite partition for five days. Fighting was attempted only sporadically. It was observed only eight times during this period, and each combat consisted of a few throat puffs and a slight darkening of color.

REPRODUCTIVE BEHAVIOR
GENERAL DESCRIPTION

THE FOLLOWING DESCRIPTION is a qualitative one and is a reconstruction of what may be termed a "usual" or "average" behavior pattern for this species, based upon observations of 21 spawnings. Only the obvious and major variabilities are considered here.

NEST PREPARATION

The entire procedure of nest preparation is accomplished by the male and consists of cleaning a sheltered area. The duration of this process depends on the presence and reproductive state of the females in the tank. The nesting behavior is always intermittent, alternating with courtship and various phases of social behavior.

The male enters and leaves his shelter frequently, and the duration of stay within the shelter is highly variable, but the presence of a pre-ovulatory female in the tank increases the intensity of nesting activity.

Nest preparation consists of five types of cleaning movements: waving (or fanning), brushing, rubbing, scooping, and nibbling. The first of these is most frequent, observable not only within the nest but occasionally at other regions of the tank floor. It consists of a vigorous waving of the body and pectoral fins, sending up a cloud of sand for five to 10 seconds. The frequency of the waving movements is about six to eight oscillations per second. The resultant forward thrust is offset by the use of the pelvic fin disc and also by the inclination of the head so that the chin is pushed against the floor. Brushing consists of waving or weaving the body and fins at a slow rate (one to four oscillations per second) and stroking the nest surfaces with the venter, dorsum, sides, or fins. Rubbing is rare and consists of scraping the head against the roof of the nest, usually in conjunction with brushing movements. Scooping is accomplished by taking up mouthfuls of sand or small shells and carrying them to other parts of the tank. Nibbling may take place if the shelter has remains of old eggshells or algae clinging to the surface. Bits of these adhering materials are picked off and usually swallowed.

Nest cleaning is rarely thorough. The adhesive algae are never cleaned off completely, and the eggshells from a previous spawning usually decay more rapidly than they are nibbled off by the male.

In females, a similar waving and scooping can be observed, and in two cases females consistently revisited and defended shallow depressions that they had constructed. Otherwise the nesting behavior of females appears random and rarely associated with a specific shelter, although females will hide in shelters and defend them for short periods of time.

If a pre-ovulatory female is placed in a tank with a male, his nesting behavior becomes intensified and mixed with courtship, as described below.

COURTSHIP

The reaction of a male to a pre-ovulatory female is clearly signaled by a characteristic pattern of courtship behavior. The coloration of the male becomes an over-all light yellowish brown, with little or no trace of the crossbar pattern, while the chin and throat are boldly blackened (pl. 27, fig. 1).

This color pattern appears within less than half a minute after the female is placed in the tank. The male approaches her slowly, to within an inch or two, usually beside or in front of her. He then waves his body, tail, and pectorals vigorously for five to 10 seconds at a time, at the rate of eight to 12 oscillations per second. This waving is similar to that involved in nest cleaning, but the body and tail are raised from the substrate, so that little or no sand is thrown up as a result of this movement.

If the female moves away, the male follows and continues courting. Often he will nip and chase her, following as she darts away. Occasionally the male will return to the nest for brief intervals, interspersing courtship with nest cleaning behavior.

In the course of the courtship phase of reproductive behavior, the courting becomes progressively more frequent and vigorous. When the female is within sight of the nest aperture, the male goes through the courting

movements inside the nest. As the female permits closer approach, or herself approaches the nest, courting movements by the male become more rapid and frequent, accompanied by quick, spasmodic gasps and throat puffs at two- to five-second intervals.

The reactions of the female to this courting behavior are various. Early in courtship she simply darts away into other shelters, corners, and higher levels as he approaches and nips. Approximately 12 to 24 hours before spawning, the female will often take shelter in the male's nest, from which she is quickly chased. However, the male, upon seeing the female within the nest, will approach with courting movements prior to entering and chasing. Occasionally a female will actually approach a male that is courting her. This occurs more frequently if the male is going through courting movements within the nest. The latter behavior appears to attract other females in the tank. As they approach closer, the male darts out and chases them and resumes courting elsewhere in the tank.

When a pre-ovulatory female approaches a nest so occupied by a courting male, not only will the vigor (amplitude and rate) of the courting movements increase, but he will frequently move over the entire inner surface of the nest, rubbing or stroking his genital region over the surface with side-to-side body movements. At this time, if water is withdrawn from the nest by means of a long pipette, microscopic examination shows no sperm to be present. A distinct erection of the male urogenital papilla is visible at this time, with this organ enlarged, reddened, and projecting at an acute angle from the body.

PRE-OVULATORY FEMALES, APPEARANCE AND BEHAVIOR

A female, a few days prior to spawning, can be recognized as being pre-ovulatory on the basis of several criteria.

ABDOMINAL DISTENTION: A distinct enlargement of the abdomen is visible, and is produced by the increase in ovarian size. Plate 25, figures 1 and 2 show a pre-ovulatory female, about 24 hours before spawning. A male is shown for comparison in plate 25, figure 1. A swollen abdomen, however, does not always indicate a pre-ovulatory state, since these fishes regularly eat large quantities of food, gorging themselves until their abdomens bulge distinctly. This enlargement disappears within 24 hours, i.e., before the next feeding, but a distention due to enlarged ovaries will of course remain.

EXTRUSIBLE EGGS: By squeezing the abdomen, eggs can be forced out from the urogenital papilla aperture. In early stages, i.e., three to four days prior to spawning, the eggs are extruded with difficulty and they are small (about one-half to three quarters of the size of mature eggs) and a pale white color. The chorions of such eggs do not become expanded in sea water, and adhering remnants of follicular tissue indicate that they have not been ovulated. As spawning approaches, the eggs become more easily extruded, larger, and yellow with accumulating yolk. About 24 hours or less before spawning, eggs can be extruded with very gentle pressure on the abdomen. These eggs are mature, i.e., ovoid, bright yellow, averaging 0.8 mm. in length (pl. 31, fig. 1). Their chorions become normally shaped and water-hardened (pl. 31, fig. 2), and they can be fertilized artificially. Ovulation may begin at this time and most certainly is imminent.

COLORATION: Three to four days prior to spawning, females show no distinctive color patterns, but one or two days before spawning the light-colored two-bar or patternless phases are frequently observed. During spawning and often just before nest entry, the ground color of the female becomes a light tan, with four narrow, dark cross lines on the dorsum. The dark cross bars in this color phase are located on the sites of areas that are normally light colored in the other color phases. In most cases this "spawning" color pattern appears only after oviposition begins (pl. 30, fig. 1).

BEHAVIOR: One or two days before spawning, the female, instead of darting away from the courting male, may take shelter in the nest. If spawning is imminent, i.e., within a few hours, she will retreat from or follow the courting male by means of short, quick, hopping movements. This movement appears to stimulate the male into more vigorous courting behavior. If no male is present, then the pre-ovulatory female may show an abortive courting behavior towards larger females or non-responsive males. The male type of

courting coloration is not exhibited, however, and the courting movements are slow and infrequent.

UROGENITAL PAPILLA: This organ is generally flaccid and pale white, but just prior to egg deposition, it becomes erected and reddened. This may be evident as long as 24 hours before spawning (pl. 24, fig. 4; pl. 30, fig. 2).

ENTRY OF FEMALE INTO NEST

During the 12- to 24-hour period preceding spawning, the female will generally enter and leave the nest several times. The time spent within the nest and the frequency of the entries increase until oviposition finally takes place. The first entries result in the female's being chased or pushed out by the male within a minute or less, whereas the later entries are characterized by stays of an hour or more within the nest.

Two types of nest entries are observed. In some cases, the female enters the nest while the male is out (pl. 27, fig. 2). This takes place in the course of the general nipping and courting activity, as the male chases the female about the tank. Here the female darts quickly into the nest, with little or no hesitation. Her coloration is either of the light two-bar type, or an over-all light brown with slight indications of four thin, dorsal cross bands. The male quickly follows with courting movements and coloration (pl. 27, fig. 2), enters the nest, and usually nips her until she leaves.

The second type of nest entry is slower and hesitant, taking place while the male is within the nest and courting (pl. 27, fig. 3; pl. 28, fig. 1). Here the female approaches the nest with short, jerky, hopping movements, often turning to enter the nest tail first (pl. 28, fig. 2). When entering, she raises her head and puffs her throat as she moves in beside the male (pl. 28, fig. 1). The courting movements of the latter become more rapid and punctuated by occasional spasmodic gasps. The coloration of the female is usually of the light brown type with or without four narrow cross bands.

The reaction of the male to the entry of a female is at first an increased vigor of courting movements. Once a female has entered

the nest the coloration of the male becomes darkened to an almost patternless black (pl. 28, figs. 2, 3). No yellow borders on the fins, such as were observed during fighting, are present. The courting movements become less frequent and are interspersed with other types of behavior, such as throat puffing, butting, biting, and nest stroking. The throat puffing behavior, with its attendant butting and biting, is similar to fighting behavior, except that gaping and quivering movements are absent. The male pushes and butts the female farther into the nest. His bites are rarely more than light mouthings or nudges. While circling the female, the male rubs or strokes his anal region and erected urogenital papilla over the surface of the nest with most frequent attention to the upper leaning surface of the tile (pl. 28, fig. 4).

At this time water pipetted out of the nest is found upon microscopic examination to contain occasional motile spermatozoa.

The female meanwhile takes on a stiffened posture, with her head raised and throat slightly puffed. Her coloration consists of the characteristic light color, with four narrow bands. The movements of the male push the female about, but she remains stiffened and unresponsive for some time. Her genital papilla is distinctly swollen and erected. If spawning is not imminent, the female may be pushed or chased out by the male, in which case another entry would be made later.

If, however, oviposition is imminent (within one or two hours), then the butting and biting on the part of the male are followed by jerky, hopping movements of the female. These in turn are succeeded by a more rapid courting, nudging, and nest stroking behavior by the male. The latter turns a still darker color, becoming a complete velvety black.

SEQUENCE OF EVENTS IN PRE-SPAWNING BEHAVIOR

At this point, for purposes of clarification, the pre-spawning behavior in *Bathygobius* may be reviewed in the form of the following list of events:

NEST PREPARATION
  Acceptance and defence of shelter by male
  Waving, brushing, and scooping—the most frequent nest cleaning movements

Increase in frequency of nest cleaning move-
 ments if females present
COURTSHIP
 Presence of pre-ovulatory female correlated
  with male display of
  Light color with black chin
  Approach to female with waving movements
  Nipping and chasing of female
  Mixture of above behaviors with nest clean-
   ing
 Reaction of female to courtship
  Retreat (if not pre-ovulatory)
  Holding ground or approaching male
  Approach to or entry into nest
  Hopping movements
  Sometimes erection of urogenital papilla
 Reaction of male to above behavior of female
  Nipping and chasing
  Frequent returns to nest
  Nest stroking with anal region
  Erection of urogenital papilla
NEST ENTRY BY FEMALE
 Several entries during 12- to 24-hour pre-spawn-
  ing period
 Frequency and duration of nest entries increase
  as spawning approaches
 Female coloration light
 Reaction of male to nest entry
  Increase in courting movements
  Darkening of body color
  Butting and pushing
  Frequent nest stroking and release of sperm
 Behavior of female within nest an hour or two
  before oviposition
  Stiffened posture with raised head
  Hopping movements
  Lighter coloration often with four dorsal cross
   bands

OVIPOSITION

Just prior to oviposition, there is an in-
crease in the speed and frequency of the jerky,
hopping movements of the female. During
the course of these movements most of the
nest surface is traversed in irregular fashion,
over and over again, including the vertical
and inclined sides of the shelter.

Oviposition takes place during the course
of these hopping movements, and the eggs
are laid in irregular, sporadic fashion, starting
usually on the inclined surface of the nest
(pl. 29, figs. 1, 2).

The urogenital papilla of the female, which
became erected several hours before oviposi-
tion, now swells into a truncated cone about
half again as long as normal (pl. 29, fig. 3;
pl. 30, fig. 2). The erected papilla is usually
projected perpendicular to the body surface
but may even be angled forward slightly. A
small triangular area just posterior to the
papilla is also swollen and reddened. The
erection recedes slightly towards the end of
oviposition, but the swelling may be visible
for as long as 24 hours after spawning is
completed.

The urogenital papilla of the female is high-
ly active during oviposition, and it is capable
of both lateral and caudo-craniad movement.
As eggs are extruded from the tip of the pa-
pilla, this organ waves quickly from side to
side, in conjunction with movements of the
trunk. These movements are often seen to
take place without any extrusion of eggs, es-
pecially just prior to oviposition. Oviposition
in progress is shown in plate 29 (figs. 1–3) and
plate 30 (figs. 1, 2).

The eggs are extruded in small, irregular
groups, adhering to the substrate as they
emerge. Approximately 10 to 30 eggs are ex-
truded in one series. They are laid in short
lateral rows (formed by the side-to-side move-
ment of the papilla), and cover an area 3 to
6 mm. wide by 5 to 15 mm. long at each ex-
trusion. After each extrusion the female
moves to another spot and extrudes a second
group of eggs. The nest surface then is covered
by eggs in an irregular, patchy fashion.

During oviposition the jerky, hopping
movements of the female are almost continu-
ous and appear to speed up slightly in re-
sponse to the nudging and biting of the male.
These movements go on at the rate of about
two or three per second, and they are pro-
duced by short, quick flaps of the pectorals
and a slight wave of the caudal. The animal,
whose head is held stiffly uplifted, is moved
forward only slightly, if at all, as a result of
each such movement. Towards the end of
oviposition, occasional quiescent spells are
observed, but the male's pushing and nipping
usually starts the hopping movements again.
The frequency and duration of the quiescent
periods increase as the end of oviposition
approaches.

Eggs are extruded in irregular patches over
the upper nest surfaces. The numerous bare
spots are gradually filled in as more eggs are

deposited around the edges of the egg patches. The female moves over the nest surface, waving the genital papilla to and fro in zigzag fashion through the previously deposited groups of eggs. When the female reaches an uncovered area at the edge of an egg patch, a new group of eggs is extruded. No eggs were ever seen to be extruded onto covered areas, but extrusion begins as soon as a bare spot is reached.

In the nest shelters constructed of leaning tiles, the ovipositions took place almost entirely on the inclined surface. This is consistent with the observation that nests found in the field had eggs deposited mainly on the ceilings of the shelters. In some instances eggs were attached to the vertical glass surfaces of the aquarium. In some spawnings the upper surfaces of the nests became crowded with eggs, and eventually some eggs were deposited on the floor of the nest. It was noted subsequently that these eggs developed normally, and the majority of the larvae became oriented with their heads towards the free, unattached ends of the eggs. The survival and hatching of these larvae did not appear to be significantly different from those of larvae hanging from the higher levels of the nest.

The coloration of the female is usually darkened and almost patternless throughout most of the ovipository period. She becomes lighter, with the four narrow bands on the dorsum, towards the last hour or two of oviposition, i.e., returning to the color phase described above (pl. 30, fig. 1).

Oviposition may last as long as nine hours but is more frequently about six hours. Extrusions of eggs take place about once or twice per minute at the beginning and end of oviposition. During the middle two or three hours of oviposition, as many as five extrusions per minute were observed, each extrusion lasting from three to five seconds.

By measuring total surfaces covered by eggs and counting representative areas, the total number of eggs deposited was estimated. The estimates for three spawnings were, in round numbers, 15,000, 17,500, and 18,000 eggs per female.

FERTILIZATION BEHAVIOR OF THE MALE

While the female is engaged in oviposition, the male is almost constantly in motion, especially during the early and middle phases. Occasional quiescent periods were observed towards the end of oviposition.

The most frequent movement observed is substantially similar to that seen towards the end of the courtship phase and during the time when the female enters the nest. The male traverses the entire nest interior repeatedly, stroking the genital papilla over the surface by means of broadly swinging, weaving undulations of the body and tail (pl. 30, fig. 3). The male urogenital papilla does not appear to be so active as that of the female.

Interspersed between these nest stroking movements, the male shows body waving movements similar to those observed in courtship but slower and of shorter duration, i.e., averaging three oscillations per second and lasting between three to five seconds. He frequently circles the female, butting and nipping her. These nudges and bites become more frequent and stronger as the female slows down or stops her jerky, hopping movements.

The erection of the urogenital papilla becomes noticeable during the latter part of courtship. After the female has entered the nest and remained there for half an hour or more prior to oviposition, the erection of the male's papilla reaches its maximum, swelling to almost half again its original length (pl. 30, fig. 4). It becomes distinctly reddened and lifted at an angle 45 degrees to 60 degrees from its original position flat against the body. As mentioned above some sperm could be pipetted out of the nest at this time, prior to actual oviposition.

At intervals of 15 to 30 seconds, the male moves about the nest stroking the bare nest surface as well as the newly deposited eggs. Although the extruded milt is not visible, sperm can be collected at this time. Plate 30 (figs. 3, 4) shows the erected urogenital papilla of the male during fertilization behavior.

The oviposition movements of the female are not directly followed up by the male, but his random strokings of the nest surface eventually accomplish complete coverage of the eggs, although the same areas may be traversed many times. These circling movements interspersed with waving movements evidently tend to maintain a sufficiently high

and uniform sperm concentration within the nest to insure fecundation of the eggs almost as soon as the female deposits them.

The coloration of the male changes from the light courtship phase to darker phases when the female first enters the nest. As oviposition begins, he becomes almost completely blackened into the color phase of the fighting behavior, except that the yellow borders on the dorsal and anal fins are lacking. Towards the end of oviposition, his coloration is lighter, tending towards brown and tan ground colors, with indistinct cross bands.

COMPLETION OF SPAWNING

The duration of spawning varies from three to nine hours, averaging about six.

During the final hour of spawning the frequency of egg extrusions and hopping movements of the female and the waving, stroking, and butting behavior of the male become lowered. Occasional quiescent periods are observed during which both partners become inactive for several minutes at a time. The coloration of the male becomes lighter, and that of the female takes on the light brown, two-bar phase.

In response to one of the male's nudges or nips, the female leaves the nest abruptly. Her coloration remains light for several hours after spawning. Her abdomen is distinctly deflated, and her genital papilla becomes flaccid and pale. She tends to retreat, remain in corners, and to retire before the nips of any other females in the tank. Within 24 hours, the urogenital papilla returns to its normal condition, and the coloration and general behavior of the female become indistinguishable from those of other females.

SPAWNINGS OBSERVED IN THE FIELD

Two separate spawnings (nos. 19 and 20) were observed in progress in the drainage basin of Marine Studios. This basin was directly connected to the Matanzas River and contained a small population of *Bathygobius* living in the debris of oyster shells.

In spawning no. 19, the oviposition was well in progress when the shell sheltering the spawning pair was sighted. The female had a typical spawning coloration, i.e., light ground color with four narrow bands across the dorsum. The male was in his darkest

phase. Although the observer could not get close enough actually to see oviposition, the movements of the female were identical with the hopping, jerky advances seen in the laboratory. The male was very actively circling the female and exhibiting those movements which in the laboratory were found to be associated with fertilization. After this spawning had been observed for about an hour, the animals and the shell were removed to the laboratory. Spawning was completely disrupted, but the male quickly found the shell and proceeded to guard and fan the eggs until hatching. The female did not continue oviposition.

In spawning no. 20, oviposition had just begun, but the shell was so placed that the spawning was not recognized until it had been disrupted by turning over the shelter. The shelter was immediately replaced, and within less than one minute the male returned to the nest. The female approached the nest within about 10 minutes. Courtship behavior and nest entry were typical of what had been observed in the laboratory. These animals were also removed to the laboratory, with the resultant disruption of spawning. The male consumed the few eggs that had been deposited within a few hours, and the female released all her eggs in one large mass and ate them.

CARE OF EGGS BY MALE

After spawning is completed, the male protects, cleans, and fans the eggs until they hatch. His most frequent movement is fanning the eggs by an intermittent waving of the body and pectoral fins. This movement is similar to that of courtship behavior and produces a strong current of water through the nest. During the course of this fanning, the male actually strokes the eggs with his fins, particularly the posterior dorsal. Occasionally he may rub or stroke the eggs with his body or head. Rarely was he observed to mouth or pick at the eggs.

During the first day or two of incubation, fanning is frequent, i.e., one to two minutes of fanning about every five minutes. On later days, the frequency of fanning is reduced to once every 30 to 60 minutes.

The darker color phases are more evident during the first day of incubation, and light

patternless or two-barred phases are more frequently observed during the remainder of the incubation period. No special or distinctive color patterns were seen at this time.

During early incubation the male rarely leaves the nest. Occasionally he leaves to pick up a bit of food near by and returns quickly. During the last two days before hatching, foraging becomes more frequent, with the male leaving the nest for an hour or more at a time.

For several hours after the exit of the female, the male not only fans the eggs but continues to circle over them, moving his urogenital papilla as in fertilizing behavior. This behavior is observed on rare occasions throughout the incubation period and especially immediately upon the return of the male to the nest after a period of absence.

HATCHING OF THE LARVAE

The period of incubation in the laboratory is about six days if the male is undisturbed. A total of eight such hatchings were observed, with incubation periods of five and one-half days (one case), six days (six cases), and seven days (one case).

Shortly before or during hatching the male fans the eggs with a considerable increase in vigor, i.e., increase in amplitude and rate of movements. The fanning is almost continuous and is accompanied by a strong brushing action of the fins.

The hatching of the larvae once begun is rapid, and as many as 90 per cent hatch within a space of 15 minutes. The continuous fanning sends literally clouds of larvae out of the nest.

Being phototactic, most of the hatched, pelagic larvae (pl. 31, fig. 6) tend to crowd together towards the lightest section of the aquarium. The male follows them and moves into their midst, fanning strongly and scattering them. This scattering action of the male continues for a few hours, after which he no longer reacts to them.

The male does not immediately return to the nest after the hatching and dispersion of the larvae. Instead he hides in the corners of the tank, and if another shelter is provided he will remain there most of the time, returning to the original shelter for short and infrequent intervals. If a pre-ovulatory female is present, courtship and nest preparation take place in the original shelter, but otherwise no attempt is made to clean out the old eggshells.

VARIABILITY

The above description of reproductive behavior in *Bathygobius* may be considered the course of events in an "average" or "typical" laboratory situation. It was recognized that a considerable amount of variation in the behavior pattern exists, but before a quantitative analysis could be made some indication of the "normal" or "usual" pattern had to be reconstructed in order to determine where the variations exist and which phases of the behavior pattern could best be quantified. It is further recognized that what has been here called the "average" or "usual" situation was based upon subjective criteria and therefore is subject to modification with the addition of quantitative data. The importance of thoroughly analyzed behavioral variations has been well emphasized by Aronson (1949).

Although at present a complete quantifica-tion of the various behavioral acts is lacking, the following section attempts to point out certain aspects of the variability of the behavior pattern.

NEST PREPARATION

The relationship between the presence of females and the nesting behavior of the male is clear. In isolated males or in all-male groups, some fanning took place but only in connection with the establishment of shelters, and once a suitable hollow was made little additional waving took place.

The presence of females was correlated with frequently repeated waving and scooping by the male. The frequency of these nesting movements, however, was variable. The variations were most evident when individual males were compared, whereas the

TABLE 2

DURATION OF PRE-SPAWNING BEHAVIOR AMONG MALES

Spawning No.	Male	Female	Days Together Before Spawning	Duration of Nest Cleaning Prior to Spawning	Duration of Courtship Prior to Spawning
2	B	3	6	5 days	2 days
4	D	5	5	5 days	6 hours
7	B	4	7	4 days	2 days
8	D	5	7	7 days	3 hours[a]
10	A	10	8	3 days	1 day
12	E	6	7	8 hours[b]	2 hours[a]
13	B	4, 8	10	6 days	12 hours
16	D	5	10	10 days	2 hours[e]
17	E	6	8	3 hours[b]	2 hours[a]
18	C	16	5	5 days	5 days

[a] In these cases, courtship behavior began only after the first nest entry by the female.
[b] Approximately.
[e] In this case, courtship began a few minutes before the first nest entry by the female.

behavior of each individual male appeared more constant. The duration of nest cleaning prior to spawning is also variable (table 2).

Male E, for example, showed very little nesting behavior. He remained within the shelter most of the time and reacted only to females that were pre-ovulatory. Once a female approached the nest and entered, however, his spawning behavior was apparently normal.

Male D was always actively waving and digging whenever females were present in the tank. His activity was not confined to the shelter. He dug numerous shallow depressions in the sand in all parts of the tank and probably spent considerably more than half his time in these "false nests."

The nesting behavior of the other males was somewhere between these two extremes, with waving and digging occurring with noticeable frequency and confined to the nest three to six days prior to spawning (table 2).

COURTSHIP

Considerable differences were found to exist in the duration and intensity of courtship behavior among the several males observed (table 2). In all cases, courtship behavior was mixed with nest preparations, but the courting was easily distinguishable be-

cause of the distinctive color pattern (black chin) associated with it.

In males B and A, courting behavior was evident only if a pre-ovulatory female was present and generally preceded spawning by not more than 48 hours. In spawning nos. 2 and 7 (with male B), courtship was first noted about two days before nest entry by the female. In spawning no. 10 (male A) and no. 13 (male B), courtship began on the day before nest entry. In all these cases, the females had been present for several days previously.

Male E, which, as mentioned above, showed little nest preparatory behavior, showed courtship activity only rarely on the day prior to spawning. Courting was observed regularly only after the first nest entry by the female. A similarly delayed courting behavior was observed in male D, who was distinctly more active in nest preparation.

Male C was exceptionally active in courtship. The presence of any female in the tank would stimulate vigorous courtship, following, and nipping. In some instances, other smaller males elicited a short courtship reaction. The reaction of this male to a female placed in the tank was so rapid and distinct that it was decided to use this individual in a series of preliminary tests on sex discrimination in this species (see below).

The reactions of females to the courting activity of the male may be any one of the following: flight, quiescence, approach to nest, or hopping movements. Pre-ovulatory females more frequently respond with characteristic hopping motions and approach the nest, especially if the male is courting from within the nest. Non-ovulatory females usually dart away rapidly as the male approaches. A quiescent response to an approaching, courting male was observed only rarely. The degree of variability among individual females in their response to courtship is not known.

OBSERVATIONS ON SEX DISCRIMINATION

The following series of transfers and observations were performed with male C. After he had been isolated for 24 hours, a pre-ovulatory female was dropped into the tank. The reaction of the male was immediate, i.e., he approached courting even before the female had reached the tank bottom. After five minutes this female was removed and another, non-ovulatory female was substituted. The reaction of the male was identical. It was noted that in each case the female darted away as the male approached.

Substituting a male (of the same size as the original female), resulted in male C's approaching the newcomer courting vigorously. When the new male remained quiescent in response to this approach, male C darkened to a complete black within about 15 seconds and drove the smaller male off into a corner with a series of bites and butts. In about five minutes male C again courted the smaller male but changed to fighting behavior within 10 or 15 seconds. The small male was then removed, and a female was substituted. A prolonged courting reaction ensued on the part of male C. This alternation was repeated eight times, and in every case the initial approach by male C was courting, which changed to fighting if the second animal was a male. On the ninth trial, the female remained quiescent at the approach of male C. He darkened into his fighting coloration, but as soon as the female darted away he resumed courtship. The alternation was repeated several more times, with no change in the results.

A large male of similar size was then introduced. Both the newcomer and male C assumed combat colors and fought vigorously. After a few minutes the second male was removed, and a large female was substituted. Male C attacked her for several minutes. She retreated repeatedly, and the male did not change to courtship behavior until 15 minutes later. This observation was repeated four times.

Similar trials were attempted with other males, but their responses were neither so definite nor so rapid as those of male C.

Gobies of both sexes were anesthetized and suspended in the tank with male C by means of thread and fine wire. The coloration of these animals was darkened by the anesthetic (a 0.1% solution of MS-222 in sea water), producing an appearance resembling that of a pugnacious male. A pale brown color, approaching that of a pre-ovulatory or ovipositing female, was induced in some of the anesthetized animals by prior treatment with adrenaline (0.1 cc. of a 1/1000 solution injected intraperitoneally).

Anesthetized animals manipulated to approach the male elicited a darkening and fighting behavior in all cases, regardless of the coloration of the anesthetized animal.

An attempt was made to simulate the retreat behavior by manipulating the anesthetized animal with wires, but the male either ignored or attacked the "model." In a single case, after about 20 trials, the male exhibited a transient courting coloration and behavior lasting only a few seconds. This reaction was not observed again either with this male or with others tested.

As controls, other objects, i.e., lead sinkers and pebbles were similarly manipulated. The males so tested retreated into their shelters and exhibited only the two-bar, and two-bar with black chin, colorations.

Crude wooden models shaped like gobies and weighted with small sinkers were manipulated in sight of males. These models were painted with imitations of the dark pugnacity coloration and the light pre-spawning coloration.

The male paid no attention to the model until it was moved towards him, when he retreated. When the models were moved in small darts towards a male within a shelter,
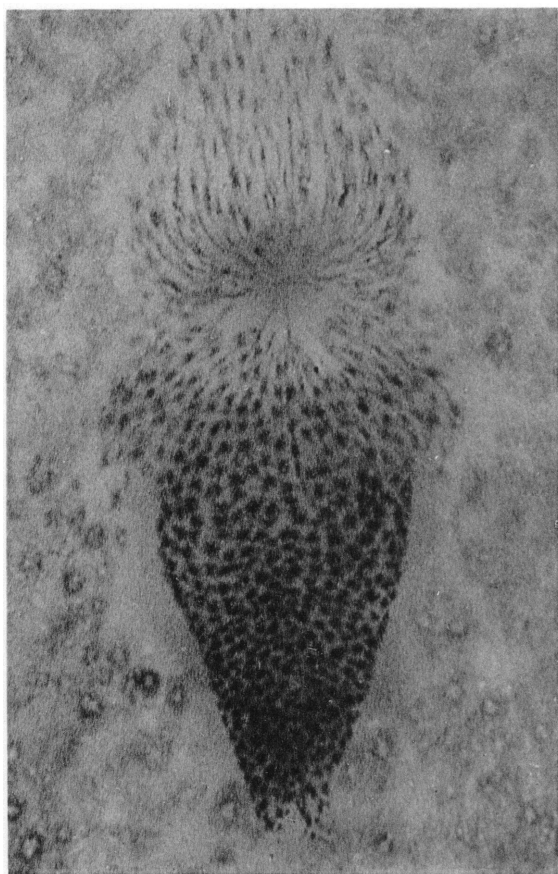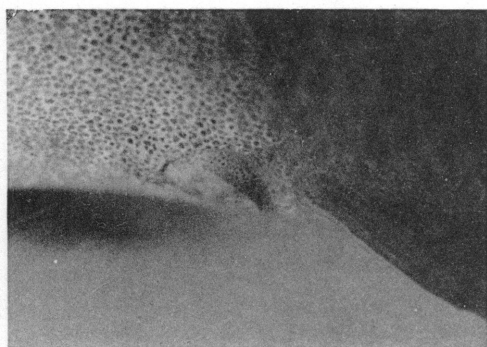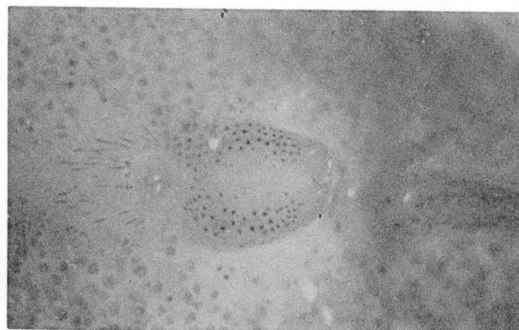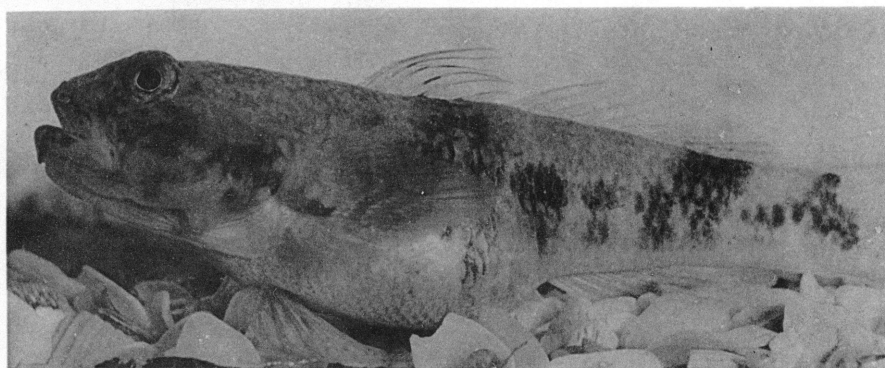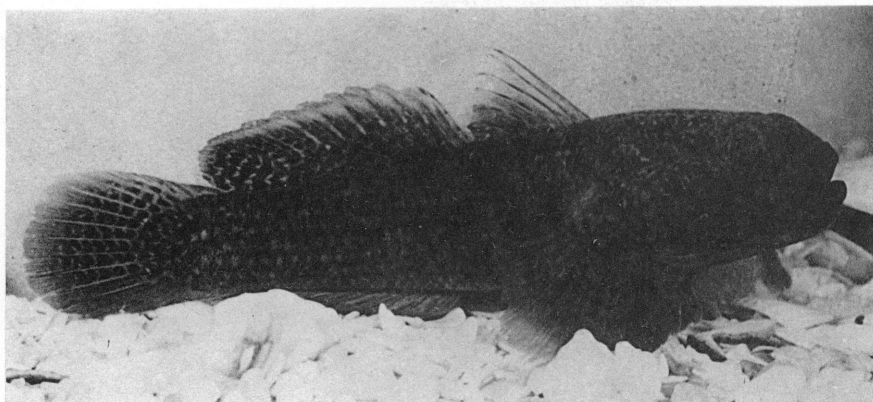
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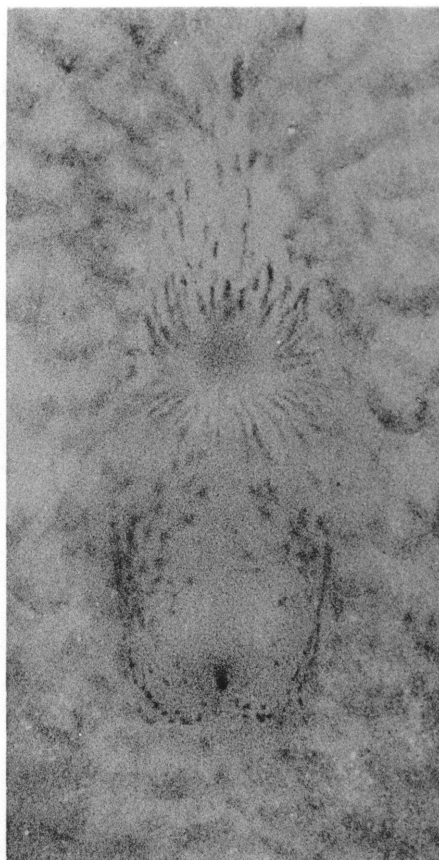
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3

4

1. Urogenital papilla of male. Ventral view. ×30
2. Urogenital papilla of female. Ventral view. ×30
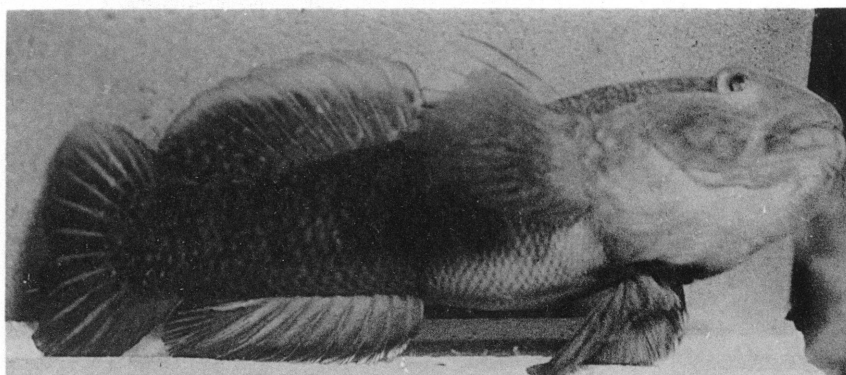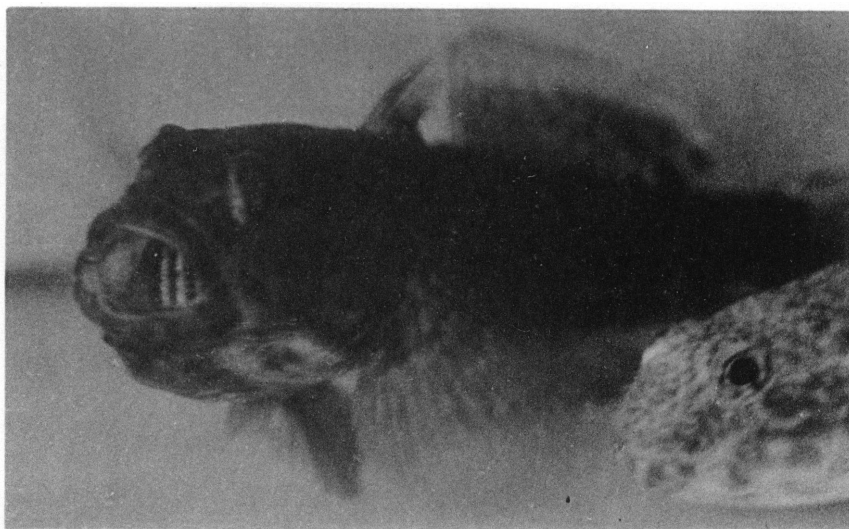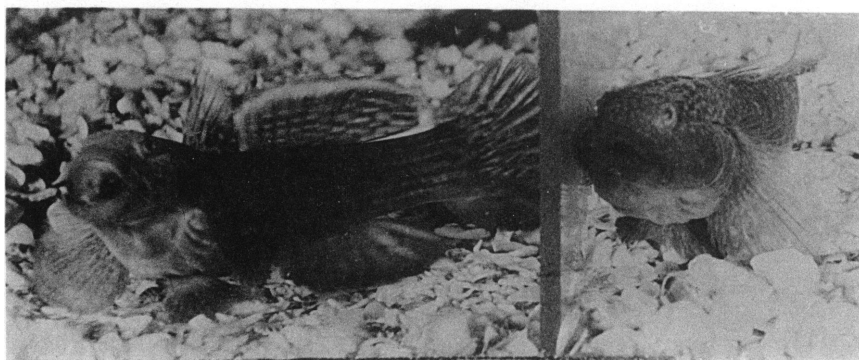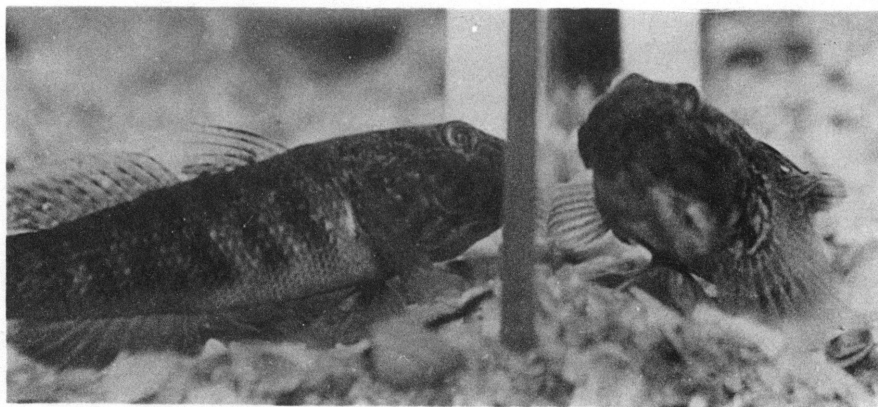3. Side view of male papilla. ×5
4. Erected female papilla. Ventral view. ×10

1. Two-bar color phase of male. ×1.4
2. Pre-ovulatory female in two-bar color phase. Note distended abdomen. ×1.8
3. Darkened color phase of male just prior to combat. ×1.2
4. Threat posture of male towards an intruding male. ×1.2
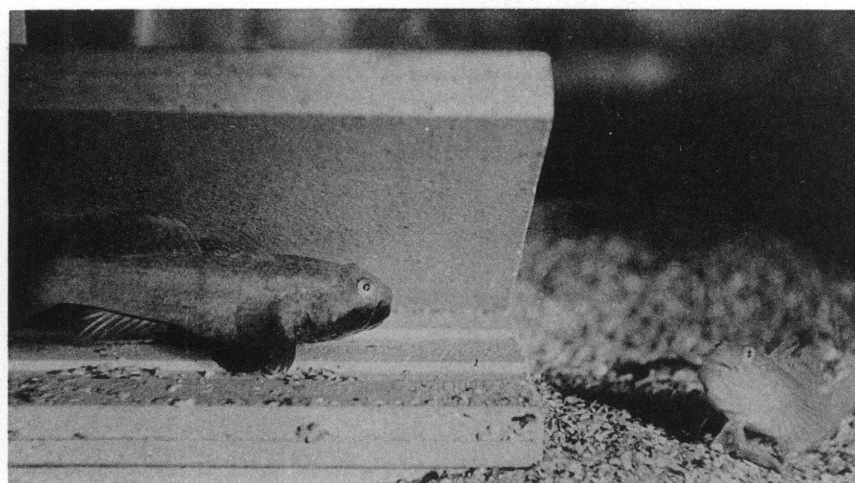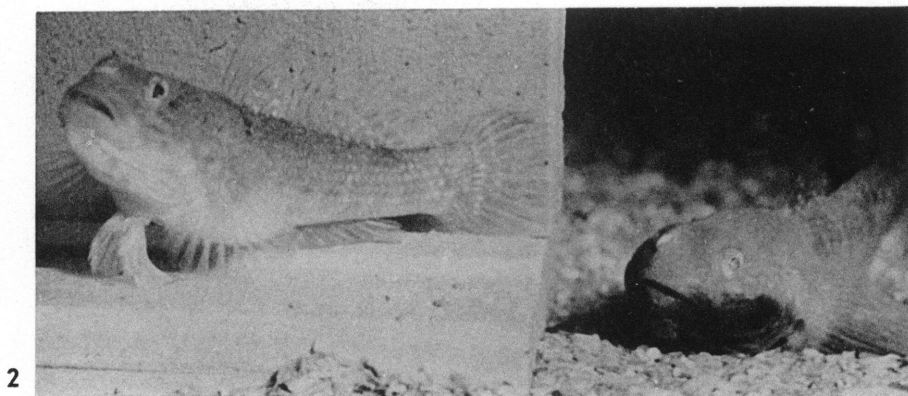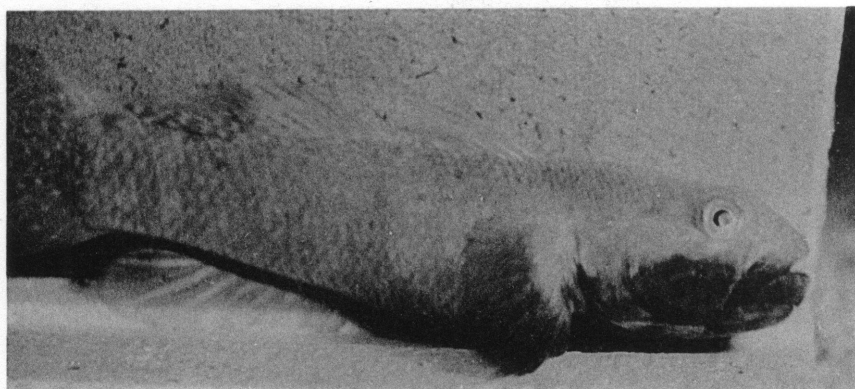
1. Darkening, gaping, and fin erection during combat between two males. ×2.0

2. Gaping, fin erection, and quivering during attempt of two males to combat through transparent partition. ×0.8

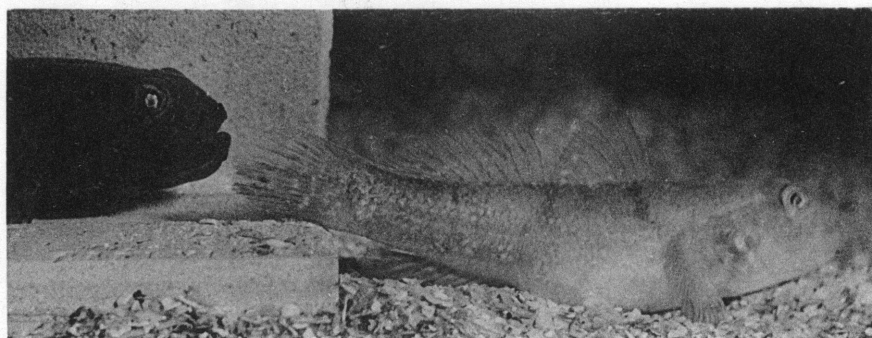3. Attempted combat between two females through a transparent partition. ×1.2

1. Courtship coloration of male. ×1.1
2. Female has entered nest and male is following (right). ×1.1
3. Female (right) is approaching nest while male (left) is courting. ×0.5
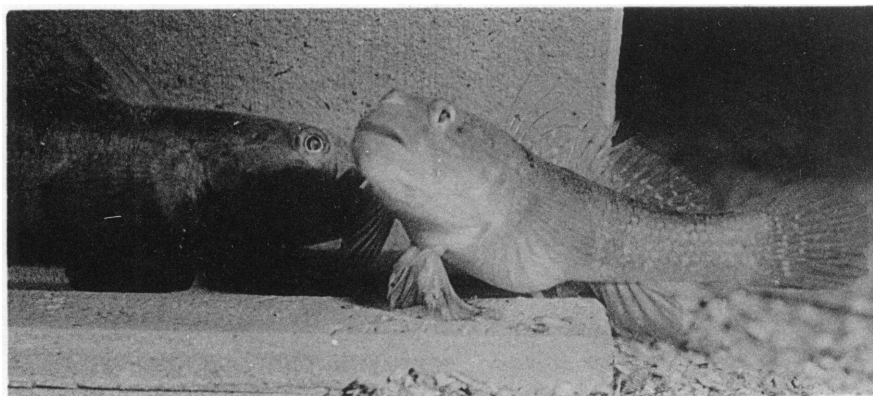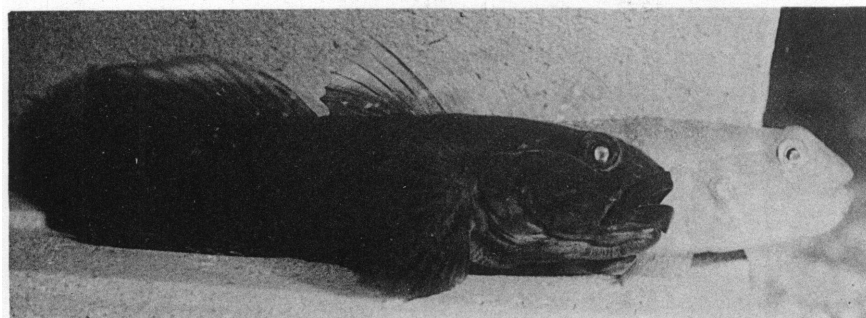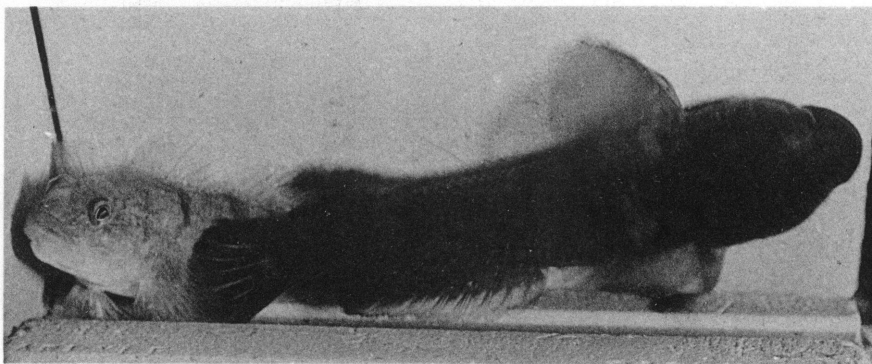
1. Female entering nest head first. Male is in "courtship" coloration. ×1
2. Female entering nest tail first. Male is in dark color phase. ×1
3. Female has entered nest. Male, in dark color phase, shows circling and tail slapping behavior. ×1
4. Nest-rubbing activity of male, in dark phase, prior to spawning. ×0.9
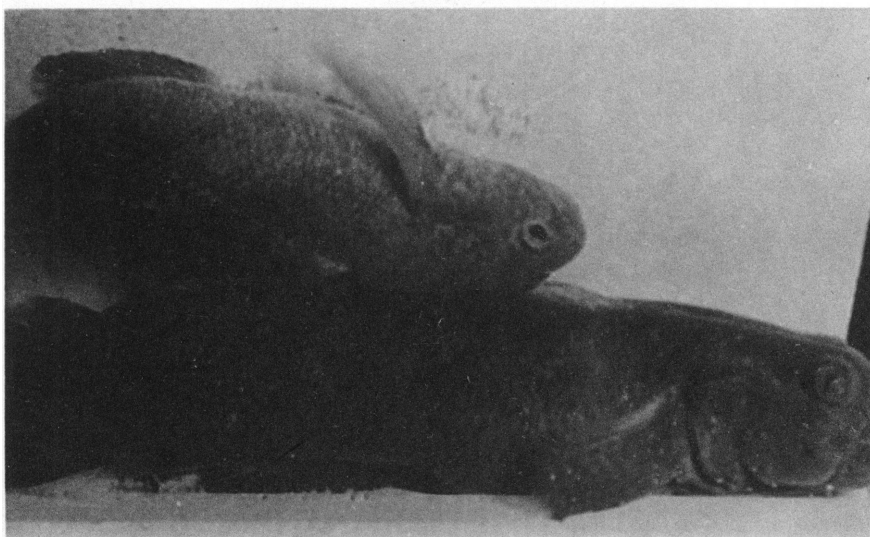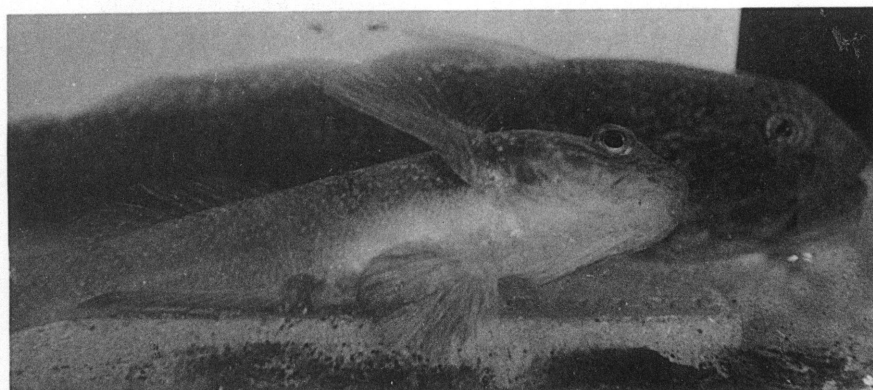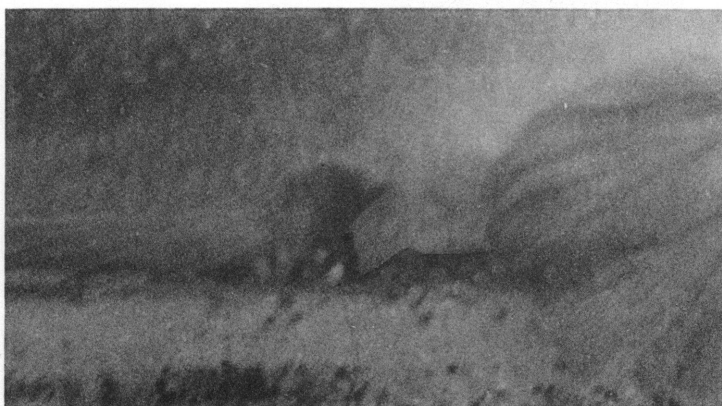
1. Oviposition has begun on back surface of nest. Note eggs adhering to nest surface just above head of female. ×1.3

2. Oviposition in progress on floor of nest. Note erected urogenital papilla of female. ×1.4

3. Enlargement of urogenital papilla of female. A few eggs have just been extruded and another can be seen within the papilla. ×5.3
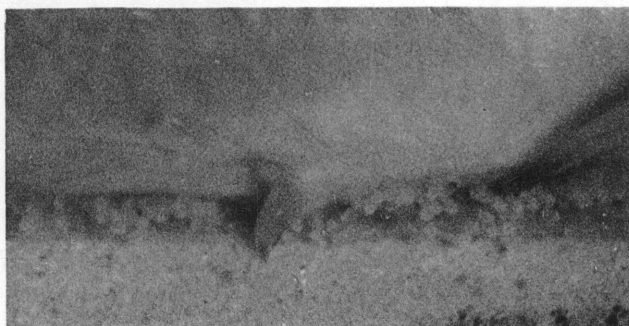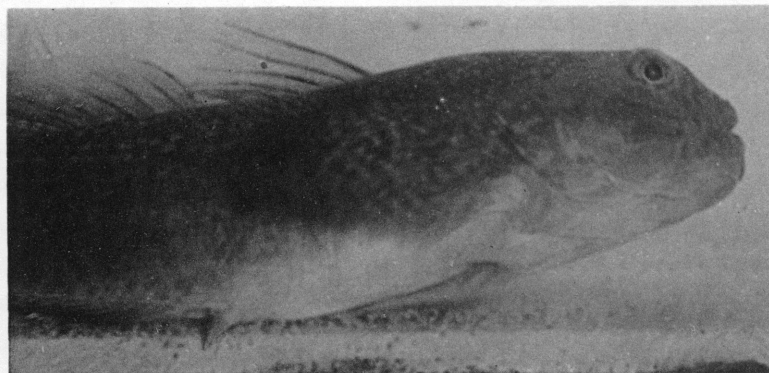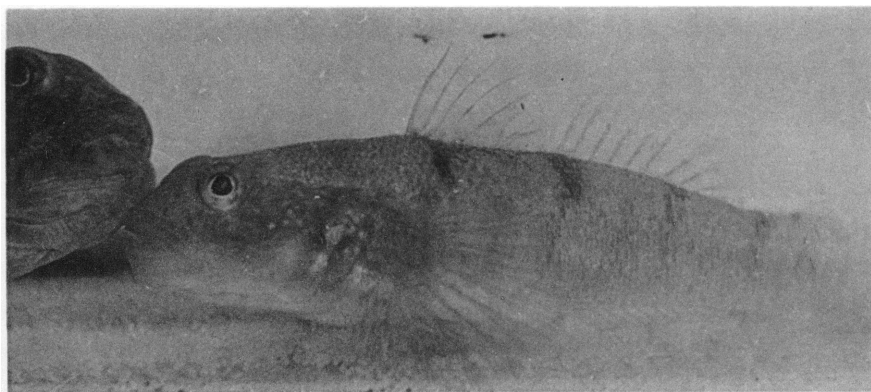
1. Oviposition is in progress on floor of nest. Female shows the "spawning" color phase. ×1.5
2. Erected urogenital papilla of female during oviposition. ×5.4
3. Fertilizing behavior of male. Note erected urogenital papilla. ×1.4
4. Enlargement of male urogenital papilla during fertilization. ×3.5
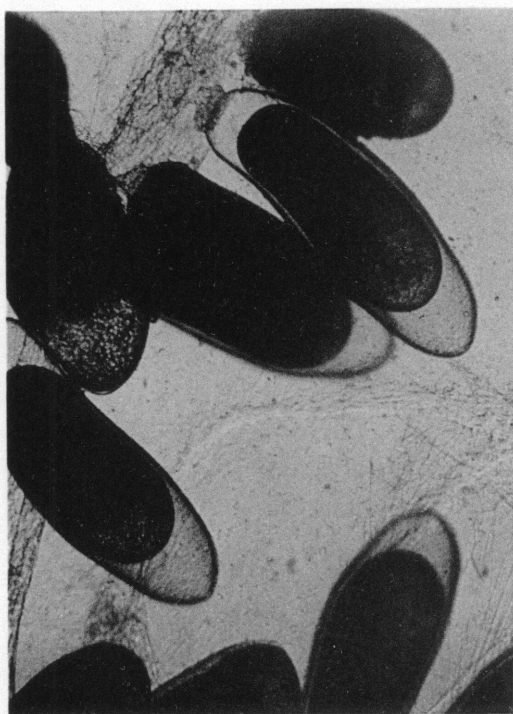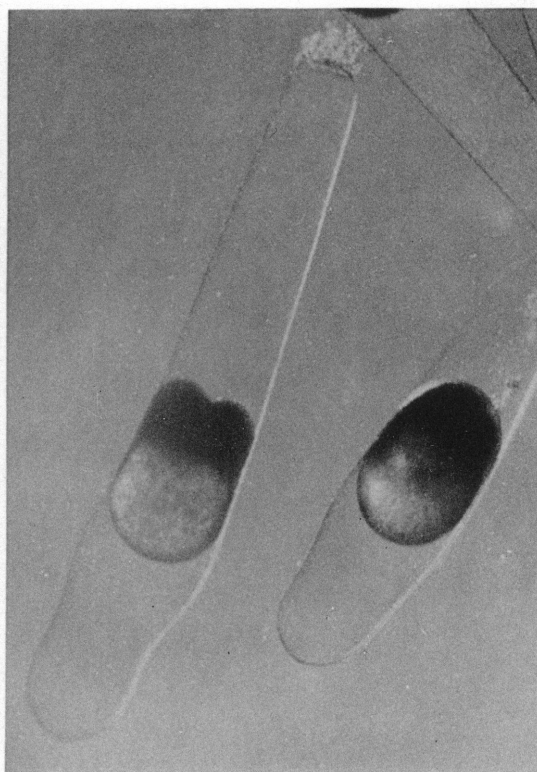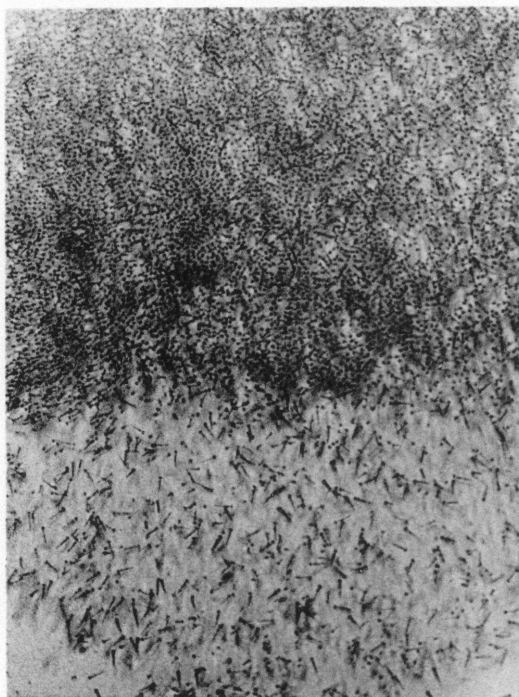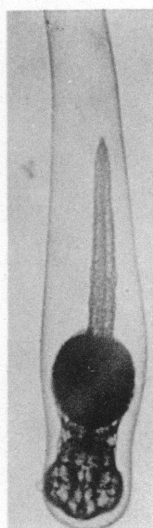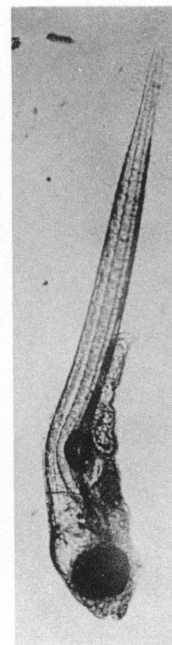
1. Freshly stripped, mature eggs. Some are already beginning to show expansion and water-hardening of the chorion. ×40

2. Normally expanded and water-hardened eggs, one of which shows the first cleavage in progress. ×40

3. Hatching of larvae. Eggs in lower half of photograph were agitated with a brush about 25 minutes before the picture was taken. ×1

4. Stage 19 embryo, representing the earliest stage at which hatching can be induced. ×30

5. Stage 20 embryo, representing the earliest stage at which over 90 per cent of hatching could be induced. ×30

6. *Bathygobius* larva, about 24 hours after hatching. ×30

he reacted by darkening and by biting the model. This continued as long as the model was kept in motion. If the motion was stopped, the male's coloration lightened to the two-bar phase, and no further notice was taken of the model. In several cases, when the model was actually moved into the shelter, the male picked it up in his mouth and carried it away in much the same fashion as males were observed to carry sand or pieces of shell during nest preparation.

NEST ENTRY BY FEMALE

This is a complex and variable behavior and is governed by several factors. The courting intensity of the male, for example, appears to affect the type, frequency, and duration of pre-spawning nest entries. With male B, in three spawnings observed, the female initially entered the nest while the male was elsewhere. She remained there, quiescent, until she was chased out. This took place without any courting behavior evident on the part of the male. After several such entries (every hour or two), the frequency of nest entries increased and the male began to show courtship movements. Eventually the female entered the nest while the male was present, took on the light pre-spawning color phase, and after only two or three such entries ovipository behavior began.

With male C, the courtship pattern was always vigorous, and nest entries by the female began two or three days prior to spawning. The nest entries were frequent and of short duration, i.e., up to three entries per hour, each of not over 10 minutes in duration. In cases where the female entered the nest in the absence of the male, his reaction, unlike that of male E, was an immediate follow-up and vigorous courting activity, which resulted in a quick retreat on the part of the female.

The reproductive state of the female is also a factor in determining nest entry behavior, since a non-ovulatory female was never observed to enter the male's nest even when prodded by the observer. The degree of variation in this phase of behavior among different females is unknown.

The length of time during the final pre-spawning nest entry prior to oviposition is a variable that is dependent on the activity of the male. In three spawnings observed with male B, the lengths of stay by the female prior to oviposition were five, six, and eight hours. In spawnings nos. 3, 5, and 14 (with male C), these lengths of stay were one and a half, three, and one hours.

During the presence of the female within the nest, male C was very active, courting, butting, circling, and nest rubbing almost continuously. Male B, however, showed the above activities only sporadically, with many periods of quiescence of up to an hour in length.

The coloration of the males during pre-spawning activity was not highly variable. The courtship color phase was evident without exception, and the darkened phase was present during and immediately preceding oviposition. The less active males (E and D) showed a change towards the "normal" two-bar pattern during their periods of quiescence.

Females showed the light color phase with four thin bars just prior to or at the beginning of spawning. In some cases this pattern was present during all the nest entries, and in spawnings with male C it appeared during the early courtship phase.

MULTIPLE SPAWNINGS ("POLYGAMY")

In four of the spawnings observed, more than one female participated in oviposition. In some cases two or more females were ovipositing simultaneously, whereas in other cases the females entered the nest and oviposited in turn.

In spawning no. 7, female 7 entered and began oviposition first. After five hours, she completed oviposition and left. While her oviposition was in progress, both the other females in the tank (nos. 4 and 8) exhibited the pre-spawning coloration and remained in the close vicinity of the nest. Within about five minutes after female 7 left the nest, females 4 and 8 entered. Female 8 was pushed out in 20 minutes, while female 4 began oviposition about an hour after entry. Female 4 completed oviposition in four and a half hours and as she left the nest she was almost immediately replaced by female 8. The latter began oviposition in one and a half hours and completed spawning in another three hours.

In spawning no. 13, female 4 entered and

began oviposition first. She completed spawning in seven hours. Females 8 and 12 showed an almost simultaneous attempt to enter the nest about two hours after the first female left. Female 8 entered and began oviposition in about two hours. Female 12, which remained just outside the nest, entered about three hours after female 8 began oviposition. About half an hour later, female 12 began oviposition; thus two females were ovipositing simultaneously. Female 8 left the nest first after an oviposition period of about eight hours, and female 12 left a few minutes later.

In spawning no. 16, the three females were all in advanced ovulatory stages when courtship by the male began. The three females entered the nest within a few minutes of one another, and all three were ovipositing simultaneously within less than an hour. It is not known in this case exactly how long oviposition lasted. It began about 10:00 P.M., and by 6:00 A.M. it had been completed.

The beginning of spawning no. 17 was not observed, but both females were ovipositing simultaneously and left the nest within a few minutes of each other.

The maximum time span during which the male will permit females to spawn is not known, but on the day after spawning brooding becomes the main occupation of the male, and no additional females, regardless of ovulatory condition, are permitted to approach the nest.

EFFECTS OF DISRUPTING SPAWNING

Spawning nos. 2, 3, 4, and 5 were temporarily disrupted by the observer's prodding and frightening the animals until both male and female left the nest. This disruption was effected shortly after oviposition had started, and it was repeated in two cases about two hours later.

In each of the above-mentioned disruptions, spawning was resumed within less than half an hour. The male returned to the nest almost immediately and courted vigorously from within it. All females in the tank approached the nest and were nipped and chased. The spawning female, however, did not retreat before the nips of the male but entered the nest by means of quick, short hops. Oviposition was resumed in a few minutes.

In the cases of spawning nos. 18 and 21, the females began oviposition with one male and were then removed and placed with a second male. In both these cases, the females so transferred were obviously aggressive, i.e., the nest was approached and entered within less than one hour after the transfer. In spawning no. 21, the second male (C) reacted immediately to the female with courtship behavior. In spawning no. 18, however, the second male (B) did not show courtship coloration or behavior until after the female had entered the nest. In the latter case, courtship was short, lasting only a few minutes, after which the male darkened and began circling and butting. In spawning no. 18, oviposition was resumed in one hour. In spawning no. 21, oviposition was resumed in about 20 minutes.

Some spawnings were completely disrupted. The two spawnings observed in the field (nos. 19 and 20) were not continued after transfer to the laboratory of both animals and their shelters. Spawning no. 19 was more than half completed, when discovered, and as noted above, the male incubated the eggs until hatching in the laboratory, while the female resorbed the remainder of her eggs within 48 hours. The female of spawning no. 20 had barely begun oviposition when she was removed to the laboratory. The following day she was still distended with eggs. She was then sacrificed and dissected. It was found that the ovaries were small and white, containing only young ovocytes, but her digestive tract was packed tightly with eggs, showing that she had released and subsequently eaten her eggs.

Spawning nos. 21 and 22 were disrupted shortly after oviposition began, and the females were isolated in small tanks. Female 12 released all her eggs in a single large mass in about 12 hours and ate part of them. Female 4 released her eggs in small clumps about 24 hours later, but did not consume any.

"FORCED" SPAWNINGS (NOS. 22 AND 23)

Females 4 and 17 were kept isolated until it became evident that ovulation was imminent (cf. p. 437). In each case the female was prodded into a nest occupied by a male, and the aperture of the nest was blocked. In both cases the male showed a darkened color

phase and began biting and butting as with an invading male. Within several minutes the female assumed her spawning coloration and began the characteristic hopping movements. The male, remaining in the dark phase, began circling and stroking the nest surfaces with his genital papilla. Motile sperm could be pipetted out of the nest at this time. The male showed no courtship behavior at any time. Oviposition began in less than an hour in both cases.

FREQUENCY OF SPAWNING IN FEMALES

Eight of the females used in these observations spawned more than once. The following table summarizes the spawning frequencies and intervals between spawnings for the various females. The spawning numbers and the female numbers are given. The inter-spawning intervals that are starred refer to cases where the females were isolated between spawnings until ovulation appeared imminent, i.e., they were checked daily for extrusible, mature eggs. The non-starred in-

tervals refer to cases where the females remained with the males.

The average inter-spawning interval is 10.6 days. The average for isolated females is 11.2 days and for non-isolated females 9.8 days. This difference is of little or no significance with so few cases. It is evident, however, that the isolation of a female does not seriously inhibit her ovarian cycle.

Maintaining females in isolation through their ovulation period did not result in their releasing any eggs, but within 48 hours the abdominal distention disappeared and upon dissection the eggs were found to be in the process of resorption.

Ovaries of females at various stages in this cycle were preserved for future histological study. It may be noted at this point that individuals as small as 40 mm. in standard length may be sexually mature. Spawning no. 9 involved a 45-mm. male and a 40-mm. female. Histological evidence (in preparation) may indicate that even smaller specimens are sexually mature.

FEMALE NOS.	SPAWNING NOS. AND INTERVALS
3	No. 2–15 days*–No 5
4	No. 3–16 days –No. 7–10 days–No. 13–12 days*–No. 22
5	No. 4– 7 days –No. 8–10 days–No. 16
6	No. 6– 7 days –No. 12– 8 days–No. 17
8	No. 7–10 days –No. 13
10	No. 10– 7 days*–No. 14
11	No. 11–10 days*–No. 17
12	No. 13–12 days*–No. 21

OBSERVATIONS AND EXPERIMENTS ON THE HATCHING
OF LARVAE

FIELD NOTES

A TOTAL of seven brooding males with their nests was collected. The nests were empty oyster shells, the inner surfaces of which were heavily coated with eggs. In five of the cases the nests contained late-stage embryos estimated at three and one-half to four days of age (Stage 21, Tavolga, 1950b). In the other two cases, the embryos were in cleavage and gastrulation stages.

The male and his nest were in each case placed together in a separate bucket of sea water. At some time during the return trip to the laboratory, the majority of the larvae were found to have hatched and were seen in large numbers floating in the bucket. This occurred in each of the five cases where the embryos were in late stages of development. Similar observations were made by the writer when collecting these gobies in 1948 and 1949 at Bimini Island, British West Indies.

The frequency of such observations seemed to indicate more than sheer coincidence, especially since the behavior of the males in the laboratory appeared to influence hatching (see below). The following facts were collated as possibly having a bearing on this phenomenon:

1. The collection of the male and the transfer of the nest to the bucket obviously produced a considerable disturbance and turbulence.

2. The collections were made at low tide in tide pools where the water temperature reached 39° C. Shortly after transfer of the specimens to the bucket, fresh, cooler sea water was added to prevent overheating during the trip back to the laboratory (one-half to three-quarters of an hour).

3. The mass hatching of the larvae was observed not less than about half an hour after the collection and addition of fresh sea water.

HATCHING OF LARVAE

Four batches of eggs were obtained by stripping gravid females, adhering the eggs to a glass slide and fertilizing them artificially by means of a suspension of finely minced testes in sea water, as described by Tavolga (1950b). These larvae developed normally up to about the fourth day, when many of the eggs were invaded by fungus growths. Approximately 25 per cent of the embryos died before hatching. Hatching began at about the fifth day after fertilization. The majority of the larvae were hatched by the sixth day, and as many as 10 per cent of the survivors hatched on the seventh day. A similar gradual hatching was observed when normally spawned embryos were taken away from the male.

It may be noted, at this point, that the developmental rate of *Bathygobius* embryos studied at Marineland was slower than that reported from the Lerner Marine Laboratory at Bimini, British West Indies (Tavolga, 1950b). The laboratory temperatures at Marineland were about 3° C. to 5° C. lower than those at Bimini, and this difference may account for the slower development. At Marineland at 26° C., Stage 18 (1.4 mm.) was reached at about 48 hours (36 hours at Bimini), Stage 20 (1.9 mm.) at about 80 hours (60 hours at Bimini), and Stage 22 (hatching, 2.3 mm.) at five days (96 to 100 hours at Bimini).

The eggshells of *Bathygobius* are shaped to fit the pre-hatching larval stage closely, and the shells are attached to the substrate at their narrow, posterior ends (Tavolga, 1950b; Breder, 1943). Thus, any wriggling movement of the contained larva tends to force the head against the rounded, free, micropyler end of the shell. Larval activity increases on the fourth and fifth day, and repeated snapping movements of the tail are visible at frequent intervals (pl. 31, fig. 5). Hatching of individual larvae is preceded by a sudden tail lashing and wriggling for five to 10 seconds. The rupture of the chorion occurs too rapidly to be seen, but the portion of the shell immediately around the head disappears and the head of the larva protrudes. With an additional snap of the tail, the larva emerges completely and darts away. The ruptured end of the chorion does not appear

torn but rather as if it had dissolved or crumbled. The remainder of the chorion remains unchanged.

STIMULATION OF HATCHING OF LARVAE

It was found that embryos as early as Stage 20 (pl. 31, fig. 5) could be induced to hatch by brushing the eggs with a soft, camel's-hair brush, i.e., simulating a strong fanning by the male. A brisk brushing for one or two minutes was required, and the reaction was not immediate. No increase in larval activity could be observed after the brushing, and the periodic wriggling and tail snapping continued as before. Within 20 minutes after the brushing, hatching of the larvae began, and by 30 minutes 70 per cent to 80 per cent of the larvae hatched. Almost all the larvae were hatched within 35 minutes after brushing. Plate 31, figure 3, shows a portion of a tile on which eggs had been deposited. The larvae were at Stage 22 (five and one-half days). The lower portion of the eggs was brushed 25 minutes before the photograph was taken. These larvae are about 80 per cent hatched. The larvae above were not brushed, and they did not begin to hatch until about 12 hours later, when they hatched gradually over a period of 18 hours.

The amount of brushing was not accurately measured, but one to two minutes of steady brushing at about eight strokes per second appeared to be sufficient. Less than one minute of brushing produced only 10 per cent to 20 per cent hatching. Brushing for as long as 10 minutes did not increase the speed at which hatching took place, i.e., initial hatchings were observed about 20 minutes after brushing was begun.

In order to test the possible effects of other stimuli upon hatching, the following treatments were applied to both laboratory spawned and artificially fertilized Stage-22 embryos: passing a strong stream of sea water over the eggs for several minutes; lowering the water temperature from the normal 26° C. to 21° C. within about one minute by the addition of refrigerated sea water; rapidly raising the water temperature to 31° C. by the addition of heated sea water; raising and lowering the tile with its attached eggs in and out of the water several times.

None of the above treatments had any effect in accelerating the hatching of larvae.

STIMULATION OF "HATCHING BEHAVIOR" IN MALES

Prior to the hatching of the larvae, males fan their eggs intermittently and slowly. About five seconds of fanning every five to 15 minutes at a rate of two or three oscillations per second was observed to be a maximum.

Prodding or frightening the male, transferring him and his nest to another tank, a strong stream of sea water, a rapid increase in water temperature from 26° C. to 31° C., and a gradual decrease of water temperature to 21° C. over a period of half an hour had no effect other than possibly reducing the frequency with which he fanned the eggs. A rapid lowering of the water temperature to 21° C. within one minute by the addition of refrigerated sea water stimulated an immediate increase of fanning behavior. This consisted of a rapid and vigorous movement lasting for one to three minutes, with a rate of eight to 10 oscillations per second. With the aid of a thermometer with its bulb placed in the nest, it was noted that the fanning began when the temperature reached 23° C. The fanning was repeated once or twice, with short five-second rests, after which the male became quiescent.

Within about 20 minutes after the above fanning was stimulated, hatching of the larvae began and was completed in 10 minutes. As soon as larvae were observed to swim out of the nest, the male resumed fanning rapidly and frequently. The duration of each fanning was not more than five or 10 seconds, with 10- to 30-second rests. The behavior of the male at this point was similar to that described above for hatchings that took place spontaneously.

The strong fanning of the male induced by the lowered temperature took place regardless of the stage of development of the embryos. Fannings were stimulated in this way when the embryos were in cleavage, gastrulation, and early tail bud stages. Hatching did not take place in stages earlier than Stage 19 (1.6 mm.; pl. 31, fig. 4). The hatching of Stage-19 embryos was obviously premature, and fewer than 30 per cent of them actually

hatched successfully. Hatching was almost 100 per cent in cases where the embryos were of Stages 20 to 22.

A fanning reaction of the brooding male was also produced by the introduction of free-swimming *Bathygobius* larvae (pl. 31, fig. 6) into the nest by means of a long pipette. The fanning was rapid but short-lived, i.e., lasting for only a few seconds until all of the introduced larvae were swept out. The introduction of other small organisms, however, failed to elicit any fanning reaction on the part of the male. Larvae of a blenny (*Chasmodes* sp.), an octopus (*O. vulgaris*), and the brine shrimp (*Artemia*) were available in large numbers and were used as substitutes for the goby larvae. The male remained quiescent or slowly fanning in their presence.

The fanning stimulated by presence of goby larvae was in no case of sufficient length or strength to produce any subsequent hatching of the male's own brood.

DISCUSSION

THE TERRITORIAL AND REPRODUCTIVE behavior of *Bathygobius* is very similar to that described for a number of other species of gobies, but most of the earlier descriptions are incomplete and based on one or two spawnings, with only a few specimens involved. Nevertheless the similarities are quite evident.

It is well known that animals occupying similar ecological niches often possess convergent similarities not only in morphology but also in behavior patterns. Many of the Blennioidea, although not closely related to the gobies, live in shallow waters and tidal zones much as the gobies and indeed may often be found inhabiting neighboring shelters. The reproductive behavior of a number of blennies has been described and, as referred to below, shows distinct similarities to that of the Gobioidea.

More distantly related teleosts residing in such environments also show a resemblance to gobies in their behavior. The Beau-Gregory (*Pomacentrus leucostictus*) possesses a strongly territorial, pugnacious, and shelter-seeking behavior (Breder, 1950). The spawning of this species (Brinley, 1939) and of the related *P. leucorus* (Breder and Coates, 1933) involves nesting, oviposition, and brooding similar to that of the gobies.

Although the embryonic development in the toadfishes (Batrachoididae) is unique in some respects, the reproductive pattern approximates that of *Bathygobius* and has been described for *Opsanus tau* (Gudger, 1908), *O. beta* (Breder, 1941a), and *Porichthys notatus* (Arora, 1948).

Numerous non-intertidal fishes also show similar behavior of nesting in cavities, including certain cichlids (Baerends and Baerends-Van Roon, 1950) and silurids (Breder, 1935).

PUGNACITY

Pugnacity, a feature common to gobies and most territorial fishes, is expressed in *Bathygobius* as a simple type of nipping and chasing behavior and also as the more specialized and complex form of combat behavior between males. Guitel (1892) described a very similar darkening, throat puffing, gaping, and fin stiffening in *Gobius minutus*. Breder (1942) reported that males of *Gobiosoma robustum* exhibit darkening and fin spreading as intimidation mechanisms. In *Bathygobius* background matching is a well-developed characteristic involving rapid and extensive color and pattern changes (Beebe, 1931; Tavolga, 1950a), but background contrasting colors are as a rule displayed only by a territory-holding individual (Breder, 1949).

Gillichthys mirabilis, the long-jawed goby, has an intimidation behavior which consists almost entirely of a display of its huge gape, a function of the distensible maxillaries (Weisel, 1947). In the blind goby, *Typhologobius californiensis*, no particular intimidation behavior is present, but males will quickly attack one another and bite viciously, holding on in bulldog fashion (MacGinitie, 1939).

In the Blenniidae, Guitel (1893) in *Blennius sphynx* and Pieron (1914) in *B. ocellaris* both described a fin bristling and gaping behavior on the part of territory-holding males. Gaping, fin spreading, and snapping are also characteristic of *Paraclinus marmoratus* (Breder, 1939b, 1941b). No associated color changes, however, were mentioned.

Intimidation behavior is often associated with bright colors, and in most cases it is the male who holds and defends territories and nests. Noble (1938) suggested that sexual selection in some fishes evolved from intimidating devices which secondarily led to nuptial colors and behaviors.

NESTING

In *Bathygobius* the primary movement involved in nest cleaning is fanning out the sand with the tail and pectoral fins. The hollows, or "false nests," are constructed in the same manner. Carrying sand and bits of shell in the mouth is a less frequently utilized method. Essentially the same nesting movements have been described for other species of gobies and blennies, as well as for most teleosts who construct any sort of hollow in sandy substrates.

The fanning method of nest formation is probably the most widespread of the nesting

activities in fishes and has been described for the Centrarchidae (Breder, 1936), certain cichlids (Baerends and Baerends-Van Roon, 1950), and often may take place in *Ameiurus* and other silurids (Breder, 1935, 1939a).

Excavation by fanning is obviously a function of the fundamental piscine geometry and is probably the most efficient type of digging activity that the construction of the animal will permit. The position of the nest is governed by several factors. Species with adherent eggs generally seek shelter in some crevice, shell, or other situation where a hard substrate will permit the attachment of eggs. Gobies and blennies are prominent members of this group, and as is the case with most territorial fishes the male selects and prepares the redd. One exception was described by Guitel (1893) for *Clinus argentatus*, in which species the female was observed to select the nesting site and attract males by means of body quivers.

Gobius ruthensparri was described by Guitel (1895) as possessing a highly variable nesting behavior. In the field eggs were found deposited on the stems of *Laminaria* and other algae, as well as on pilings and buoys. In the laboratory, the under surfaces of shells were used as in *G. minutus* and most other gobies.

COURTSHIP AND SEX DISCRIMINATION

Some form of courting behavior is exhibited by males of most nesting species of fishes, but even among the gobies and blennies, there is considerable difference in the details of this behavior from species to species. The courting male of *Gobius minutus* (Guitel, 1892) undergoes body tremors and rapid breathing movements. He approaches the female with short hops, his fins bristling, head raised, throat puffed, mouth agape, and his chromatophores are expanded into the most brilliant color phase. The *Gobiosoma* male (Breder, 1942) approaches the female with short darts, while his fins are spread and his color is almost a complete black. Field (1945) reported the courting activity of the brackish-water *Brachygobius xanthozonus* in which the male, in lighter colors, swam back and forth in front of the female.

Among the blennies, Guitel (1893) described the courting of *Blennius sphynx* as

chasing and butting, while the male was in his brightest and most contrasting color phase throughout. The above author also described the quivering and supination of the female of *Clinus argentatus* as attractive to males. Courtship by males of this species also takes place and involves a complex series of short jerky movements, stroking, chasing, butting, trembling, and brightened colors. In *Paraclinus marmoratus*, Breder (1941b) described a male, when approached by a gravid female, as turning an intense purple color and vibrating his body. The female at this time possessed a very light coloration.

It is perhaps too easy to overrate the importance of courtship behavior in fishes. As shown in the present paper, females can at times "recognize" a male in the absence of any courting activity on his part, and the courtship of the male is not always correctly directed. Courtship and in particular nuptial colorations are not always involved in sexual discrimination and selection, nor are they necessarily involved. Noble (1938) indicated that in many cases "nuptial" colors serve a variety of other functions, such as threatening invaders and aiding in territorial dominance. However, although courtship *per se* may not be essential for spawning, the courting colors and activities unquestionably make the male more conspicuous and therefore enhance the probability of successful pairing. The response of individuals to specific items of behavior or color, i.e., to "sign stimuli," may be a learned response, as, for example, the sex recognition in male guppies (Breder and Coates, 1935; Haskins and Haskins, 1949).

Courtship may be mutually stimulating to both sexes and hence become a factor in coordinating the behavior of a physiologically matched pair. This is especially important in situations where the male and female are not matched, i.e., the female not ovulating, for example. In such cases, the courtship behavior of the male would maintain the pair until the proper physiological state of the female was attained. Clark and Aronson (1951) and Clark, Aronson, and Gordon (1949, 1954) have shown that in the viviparous poeciliids major courtship activities were highest in those pairs where copulations did not readily occur.

Once a female is attracted to an actively courting and conspicuous male, the "recognition" of a gravid female by the male may depend on her behavior and/or on her coloration. In studies on the sunfish (*Eupomotis*) both Noble (1934) and Breder (1936) concluded that the female is permitted to enter the nest primarily because she "holds her ground" and does not retreat in the face of the male's intimidation tactics. Coloration in this case appears to play no role in sex discrimination by the male. Similar conclusions were reached by Breder (1934) for the mechanism of sex discrimination in the cichlid *Aequidens latifrons*. The observations reported here on *Bathygobius*, although preliminary in nature, indicate that the male permits nest entry by a female that resists his nipping, butting, and other pugnacious activities.

OVIPOSITION

In *Bathygobius*, oviposition begins on the sides or roof of the nest, where the inverted female deposits eggs. Eggs are placed on the floor of the nest only after the other surfaces have been completely or partially covered. Tavolga (1950b) found that, unless artificially fertilized eggs were placed in a hanging position, many of the larvae did not rotate properly within their elongate egg shells. Such individuals developed with their heads pointed towards the attached end of the shell and were thus unable to hatch. In the present observations it was found that the majority of the larvae on the floor of the nest developed and hatched normally. It is probable therefore that the fanning activity of the male in some way prevented the abnormal positioning of such larvae and that, contrary to the earlier idea, the orientation of the larvae is only partially dependent upon gravity.

Oviposition on the under side of shelters is almost universal among the gobies and blennies described. Exceptional situations occur where, as here noted, an insufficient surface or other environmental factor prevents the normal supine position of the ovipositing female. Fishes that hide and spawn under shelters are in the majority of cases likely to be confronted with a nest floor which consists of sand, mud, or other irregular sur-

face, whereas the ceiling will probably possess a hard, smooth area more suitable for the adherence of eggs. The question then arises as to whether there is some survival value in the placement of the eggs on the ceiling of the nest which resulted in the natural selection for this genetically determined behavior, or whether this is a behavior pattern acquired by habituation to an environment where the upper portions of the nest are more frequently suitable for oviposition than the floors.

The course of oviposition as described here for *Bathygobius* appears to be very similar to that reported for *Gobius minutus* by Guitel (1892). Eggs are deposited by the female in small, irregularly distributed groups, and the bare spots are gradually filled in, resulting in an area completely and evenly covered with eggs. While depositing eggs, the female moves over the entire nest surface a number of times, and as she does so her urogenital papilla waves to and fro through previously deposited eggs. Since eggs are often deposited immediately upon reaching a bare surface, it seems likely that the papilla possesses some tactile function, i.e., the stimulus of the eggs already present may inhibit the deposition of additional eggs in the same spot. The urogenital papilla of the male is evidently less sensitive to touch, since the presence of eggs is not essential for the release of sperm.

A similar technique of oviposition, where the female "feels" for vacancies, was described for *Aequidens latifrons* (Breder, 1934).

"POLYGAMY"

The fact that a number of females may contribute to an egg mass fertilized and guarded by a single male is known for a wide variety of nesting fishes. It is often deduced from the presence of groups of eggs at different stages of development found in the same nest.

In *Bathygobius*, the females may spawn simultaneously or take turns. Because the male will accept no additional females 12 to 24 hours after spawning, the embryos are not more than a day apart in age, and hatching of all the eggs can take place at the same time.

The ability of a spawning female to continue spawning with a second male after being transferred to another tank indicates

that in the field it is quite possible that a female may spawn with more than one male if the original spawning is disrupted or the shelter becomes too crowded with eggs. Guitel (1892) described females of *Gobius minutus* as spawning with more than one male. In the blenny, *Clinus argentatus* (Guitel, 1893), the pre-spawning quivering activity of the female may attract several males in the vicinity.

Males of *Gobius ruthensparri* were reported by Guitel (1895) to accept females throughout the brooding period of a previous spawn. As a result the male in many cases may be brooding eggs continuously through the breeding season.

The unnatural crowding which may exist in laboratory conditions could cause such "multiple spawnings" to take place, although they might not occur normally in the field. The finding of eggs at different stages of development would support the probability of more than one female's contributing to the nest. Although such nests have not been found in the case of *Bathygobius*,[1] they have been reported frequently in other ecologically related species. It has been pointed out (Breder, 1948) that small aquaria tend to simulate tide pool conditions. It is likely therefore that laboratory observations on "multiple spawnings" are indicative of a normal situation rather than one entirely fostered by an artificial environment.

SPAWNING FREQUENCY IN FEMALES

Among *Bathygobius* females, spawning frequency in captivity averaged about once every 10 days, but the variation among the eight females timed was considerable, indicating that further data are needed. Although with the present information the difference in spawning frequency between isolated females and those that remained with males was not significant, it would be of interest to determine what effect, if any, the presence of another animal would have on the ovarian cycle.

[1] After this report was sent to press, one such case has been found. The embryos in this nest were in three stages of development: gastrula (Stage 9), tail bud (Stage 14), and Stage 20. The late stages were distributed irregularly towards the periphery of the redd, with the gastrulae in the center.

Noble and Curtis (1939) in *Hemichromis bimaculatus* and Aronson (1945, 1951a) in *Tilapia macrocephala* found that in these cichlid fishes the visual stimulus of another fish, particularly a brightly colored one in *Hemichromis*, stimulated pre-spawning behavior and ovulation. Ovulation may also take place in isolation (Aronson, 1945, 1951a). Further investigations of this sort in *Bathygobius* are indicated.

Little data are available on the spawning frequency in gobies and blennies. Guitel (1892) gave an average seven-day interval for *Gobius minutus* and (1895) a six-day period for *G. ruthensparri*. These figures fall within the range of variation for *Bathygobius*. MacGinitie (1939) stated that *Typhlogobius* usually spawns once during a reproductive season.

NEST CARE

In the majority of nesting fishes, it is the male who broods the eggs. Among the gobies, only *Typhlogobius* is reported as an exception in that both sexes guard and fan the spawn (MacGinitie, 1939). The crested blenny (*Anoplarchus p. purpurescens*) of the Pacific coast is the only blenny reported in which the female guards the nest (Schultz and DeLacy, 1932).

In *Bathygobius*, the brooding behavior of the male consists almost exclusively of fanning, and little if any mouthing of the eggs was observed. Some type of fanning, using some or all of the fins, is present in most nesting fishes. The butterfish (*Pholis gunnellus*) is an exception in that, although the parents remained coiled about the egg mass, no fanning was observed (Gudger, 1927). An example of highly developed nest care was described by Breder (1941b) for *Paraclinus*, in which the male not only fanned and brushed the eggs but frequently shook and fluffed them with his mouth.

The function of the brooding is two-fold, i.e., aid in water circulation for respiratory purposes and prevention of growth of bacteria and molds. It is a well-known fact that one of the difficulties in raising the eggs of nesting fishes artificially is their tendency to be killed by fungal growth. The importance of mechanical agitation, as well as the possible bactericidal properties of the mucous

secretions of the parent, has been brought out by Shaw and Aronson (1954). Although their investigations concerned the survival of embryos of the oral incubating cichlid *Tilapia macrocephala*, a similar protective action may exist in those species that brood their eggs by fanning.

THE ROLE OF THE MALE IN THE HATCHING OF LARVAE

Hayes (1942, 1949) investigated the mechanisms of hatching in salmonid fishes. He described an initial softening of the chorion (the "Hein effect") and the later effect of the hatching enzyme. The latter was considered as the main, if not the only, factor in the actual breakdown of the eggshell. The digestion of the chorion by the hatching enzyme did not dissolve the egg capsules completely, but they "became gossamer ghosts of their former selves which vanished on shaking into a suspension of microscopic particles" (Hayes, 1942).

The crumbling of the chorion was observed in *Bathygobius*, but evidently the effect of the hatching enzyme was restricted to the region directly around the head of the larva, since the remainder of the shell did not appear weak or crumbly. In salmonids, the movements of the larva were of little consequence in the hatching process, but as a result of the presumably localized action of the enzyme in *Bathygobius* and the peculiar, elongate shape of the egg the larval tail lashing is essential if the animal is to break through the weakened chorion and wriggle out.

The fanning and brushing activity of the male in the hatching process was found in the present work to have two distinct phases: (1) a preliminary, strong, extended brushing which is stimulated by a drop in water temperature; and (2) a secondary fanning which is associated with the presence of hatched larvae in the nest. The first phase resulted in hatching within about 30 minutes whereas the second fanning alone did not stimulate hatching. For purposes of distinction, the first, longer, more vigorous fanning is referred to as "brushing," as distinguished from the second, shorter "fanning" of the larvae out of the nest.

The brushing may have any of several pos-

sible functions, among which would be: the breakage of the chorions already weakened by the hatching enzyme; the stimulation of the larvae to greater wriggling activitiy which ruptures the weakened chorions; and the stimulation of the larvae to secrete the hatching enzyme. The first two interpretations assume that the hatching enzyme had been secreted by the larvae for some time prior to the brushing. The presence of a time lag of 20 to 30 minutes after brushing, either artificially or by the male, tends to rule out the first interpretation, because if brushing broke the chorions hatching would begin immediately.

It is not clear from the present observations as to whether or not the larval movements increase after brushing. If such is the case, the increase is certainly not distinctive, because larvae of Stages 21 and 22 can be seen to lash and snap their tails frequently without prior brushing and many hours before hatching. In any case, if the egg cases had already been weakened by the hatching enzyme sufficiently for larval movements to rupture them, considerable breakage would be expected to take place during brushing.

It appears likely then that the brushing activity of the male stimulates the secretion of the hatching enzyme and that this enzyme can be secreted by embryos as early as Stage 19 (64–70 hours at Marineland). The half-hour lag before hatching would represent the time necessary for digestion of the shells. This is considerably faster than the action of the enzyme reported by Hayes (1942). He stated that the release of the enzyme is not gradual and usually appears suddenly in full strength. Hatching took place within a few hours after the release of the enzyme. Hayes also reported that the salmonid hatching enzyme operates at maximum effectiveness at 21° C., and shows a lowered digestive rate at temperatures above and below that point. If the nature of the gobiid enzyme is similar, it may well be that the same temperature drop that stimulates the brushing behavior of the male also enhances the action of the hatching enzyme.

The secondary fanning, although not effective in stimulating hatching, is instrumental in scattering the larvae out of the nest. A similar behavior has been described

by Field (1945) in males of *Brachygobius xanthozonus*, the "bumble-bee fish." As in *Bathygobius*, males of this species follow the schools of larvae and fan in their midst, scattering them widely.

The evidence presented here clearly points to the conclusion that, once hatching begins, the escaping larvae are a stimulus to the male which results in his fanning behavior. This fanning produces not only a scattering of the larvae but almost certainly ruptures eggshells that have been partially or completely digested by the hatching enzyme. In undisturbed laboratory conditions the male broods his eggs in aquaria in which the temperature remains steady, and the hatching takes place spontaneously. It is probable that in these cases enough enzyme is accumulated by gradual secretion. Once hatching begins, the male aids in its completion. It is also notable that hatching is considerably delayed in these cases.

The natural stimulus for the male's brushing behavior may be the inundation of the tide pools by the incoming tide. Tide-pool water rises in temperature to well over 30° C. in the summer, while normal water temperatures remain at least 5° to 10° lower. The inundation of a tide pool by a wave during a rising tide would lower the pool's temperature quite suddenly. Thus, in the field, hatching would take place on an incoming tide at the earliest embryonic stage at which the hatching enzyme could be secreted in any quantity. This synchronization of hatching with an incoming tide would obviously be of considerable survival value to the species, insuring a wider and more rapid dispersion of the pelagic larvae.

In the analysis of the secondary fanning, a question arises as to the degree of specificity with which a brooding male can "recognize" and react to his hatching larvae. Other larval animals of similar size and behavior did not elicit any response. It would be of interest, then, to plan experiments which would test the cues or "sign stimuli" by which the larval gobies are "recognized."

SUMMARY

THE REPRODUCTIVE BEHAVIOR in the gobiid fish *Bathygobius soporator* (Cuvier and Valenciennes) presents a pattern fundamentally similar to that of other gobioid and blennioid teleosts that live in the tidal zones. *Bathygobius* is shelter seeking and pugnacious with respect to other gobies.

Color changing is a well-developed ability in this species. Aside from the variety of background-matching colorations that can be assumed, several background-contrasting colorations are correlated with fighting and with reproductive behavior, especially in males. Extreme darkening is characteristic of fighting males. This occurs together with throat puffing, gaping, quivering, butting, and biting movements.

Reproductive behavior consists of the following sequence of events:

1. NEST PREPARATION: The male cleans out a shelter by means of fanning, brushing, scooping, rubbing, and nibbling.

2. COURTSHIP: A light coloration with a blackened chin and throat is exhibited by the male as he approaches a female and vibrates his body and tail. A pre-ovulatory female is characterized by a swollen abdomen, extrusible mature eggs, light coloration, and hopping reaction to a courting male.

3. NEST ENTRY: Twelve to 24 hours prior to spawning, the female may enter and leave the nest several times. Her coloration becomes a light brown, with four narrow dorsal cross bands.

4. OVIPOSITION: The female extrudes adherent eggs onto the walls and ceiling of the nest during the course of short, hopping movements. Her urogenital papilla becomes erected and is highly active during oviposition. About 15,000 to 18,000 eggs are produced.

5. FERTILIZATION: The male intersperses butting and nipping with an irregular traversing of the nest surface with his urogenital papilla while the female oviposits.

6. BROODING: After oviposition is completed, in three to nine hours, the female leaves and the male guards and fans the eggs.

7. HATCHING: After an incubation period of five to seven days, in the laboratory, hatching is accompanied by the fanning movements of the male.

Observations of variations among 21 spawnings showed the following:

1. Male nesting and courting activity is increased when a pre-ovulatory female is present.

2. Pre-spawning activities among males vary greatly in duration and frequency.

3. Based upon preliminary observations using anesthetized fish, models, and interrupted spawnings, sex discrimination among males appears to be a function of the behavior of the female in response to courtship.

4. The frequency and duration of nest entries by females are variable with respect to different males and different females.

5. Multiple spawnings ("polygamy") can take place in the laboratory, with as many as three females ovipositing simultaneously.

6. Courtship phases are omitted under conditions of "forced" spawnings, i.e., when the pre-ovulatory female is confined in the nest with the male.

7. Females can spawn at intervals of from seven to 16 days.

Field notes, observations on laboratory spawnings and artificially fertilized eggs, as well as experiments involving the effects of mechanical stimulation and temperature changes on brooding males and on pre-hatching larvae, lead to the following conclusions:

1. A strong brushing of the eggs by the male is stimulated by a rapid drop in water temperature.

2. The above brushing stimulates the hatching of larvae within 20 to 30 minutes, probably by aiding in the release of the larval hatching enzyme.

3. The presence of free-swimming larvae in the nest stimulates the male to further fanning and scattering activity, during which the larvae are dispersed and their hatching is further mechanically aided.

4. In the absence of a male, hatching takes place gradually over a period of many hours. Artificial brushing, however, induces hatching within 20 to 30 minutes.

5. In the absence of the temperature

stimulus, hatching is delayed, but once begun its completion is aided by the fanning of the male.

6. The natural temperature stimulus may be the inundation of the tide pool by cooler water during a rising tide. This would insure a wider dispersion of the pelagic larvae by the tidal water currents.

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