EFFECTS OF PRIOR EXPERIENCE UPON NESTING AND RESTING SITE SELECTION BY THE MOSSAMBIQUE MOUTH BROODER, TILAPIA MOSSAMBICA

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EFFECTS OF PRIOR EXPERIENCE UPON NESTING AND RESTING SITE SELECTION BY THE MOSSAMBIQUE MOUTH BROODER, TILAPIA MOSSAMBICA

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ABSTRACT

EFFECTS OF PRIOR EXPERIENCE UPON NESTING AND RESTING SITE
SELECTION BY THE MOSSAMBIQUE MOUTH
BROODER, TILAPIA MOSSAMBICA

By

Christopher L. Thoms

The purpose of this study was to determine the effect of prior experience on nesting and resting site selection in a cichlid fish. The Mossambique mouth brooder, *Tilapia mossambica*, Cichlidae, was reared over either a sand, gravel, or stone substrate from free-swimming until sexual maturity. Pairs of fish were tested in an experimental tank giving a choice of the rearing substrate and one of the other substrates. Pairs were tested until ten pairs had dug nests in either the rearing or choice substrate. Pairs which did not dig nests by the end of the eighth day following introduction were removed. The fish were divided into six experimental groups based upon rearing and choice substrate combinations. Fish were observed each morning, 60 to 90 minutes after feeding. A search for nesting sites was made each afternoon.

Males chose the smallest particle size in which to dig a nest, regardless of prior experience. It was suggested that this specificity could act as a species isolating mechanism.

Eight males dug two nests in the same substrate. The function of more than one nest in a territory is not known. It may offer a super sign stimulus to a female entering a territory.

Neither prior experience nor the type of substrate influenced the diameter dimension of a nest. The depth dimension differed among the experimental groups. This difference was due to the deep nests dug by one of the groups.

Prior experience over a particular substrate did not influence the elapsed time between introduction and the digging of a nest.

In at least nine cases, males established territories which encompassed more than one compartment of the experimental tank. In seven of the nine observed cases, the resting substrate differed from the nesting substrate.

In those experimental groups for which sand was either the rearing or choice substrate, the number of pairs not digging nests varied from three to five. The two Experimental Groups tested over gravel and stone had nine and twelve pairs not digging nests. Possible explanations for the number of pairs not digging nests were discussed. It was suggested that such pairs did not acclimate as rapidly as did those which dug nests. It was also suggested that in the absence of the preferred sand substrate, fish are less likely to breed.

The distribution of the fish was affected by an interaction between the selection of a substrate suitable for nest digging and the effect of prior experience. The importance of nest-site selection and prior experience differed depending upon the relationship between the rearing and choice substrate particle size.

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In order to test the preference of fish given two unfamiliar substrates (gravel and sand), a seventh experimental group was added to the original design. All nest site parameters of this group were similar to those of the other six groups. The distribution of the fish differed from those of the other groups. The percentage of fish over either gravel or sand fell between those of the experimental groups which had experience with one of these substrates. This is suggestive of the effect of nest-site selection without the effect of prior experience.

The results of this study were discussed in relation to previous studies of habitat selection. Recommendations for future study and applications were made.

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By

Christopher L. Thoms

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There were many people involved, either directly or indirectly, in my completion of this research project. If I have left anyone out, I hope that they will realize that it is only on paper.

My major professor, Dr. James C. Braddock, has not only put up with me for more than four years, but also given me the independence to pursue this and other research projects. His guidance in the development of my teaching and research interests has been invaluable.

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Many long discussions with my good friend, Carl Quertermus, did much to develop this project during its embryonic stages. Carl's own Ph.D. research initiated my interest in habitat selection.

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Lastly, and certainly first, I very greatly appreciate my family's patience.

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INTRODUCTION

Habitat selection is based upon the experience and the genetics of the animal. A number of investigators have examined either one or both of these components. None have examined both in relation to reproductive and non-reproductive behavior in a species that migrates to a specific breeding site. This study was designed to examine the role of prior experience upon nesting site selection by *Tilapia mossambica*, Cichlidae.

Habitat selection is the psychological choice of a habitat by an animal (Lack, 1933). It limits the distribution of a species within the limits established by predation, competition, and physiological tolerance (Harris, 1952; Wecker, 1963; Klopfer, 1969). The effect of experience with particular stimuli upon the later behavior of an animal has been investigated by Klopfer (1962, 1963, 1965), Klopfer and Hailman (1965), Wecker (1963), and Quertermus (1972). From these studies and others it has been found that experience can modify, but not change, an innate response. This is true for either early or prior experience (King, 1958). The effect of experience upon the response of an animal may differ depending upon the physiological state of the animal (Klopfer, 1965; Quertermus, 1972). It is important also to consider that a stimulus must be relevent to the genetic background of a species and the behavior being observed.

These general aspects of habitat selection will be discussed specifically in the section on reproductive and non-reproductive behavior.

Reproductive Behavior

According to Hildon (1965), certain environmental stimuli release innate reactions in birds by which suitable nesting sites are recognized. This concept would appear to be applicable to all species, although this has not been tested. Lack (1944, 1949) suggested that interspecific competition could account for the nest-site specificity in birds. The species-specific factors determining nest-site selection are poorly known as is the role of experience.

Oviposition sites are selected by many of the Insecta (see review by Krebs, 1972): site locations depend upon location of male territories for certain species of dragonflies (Macon, 1963), attractive odor of the corn plant for the European corn borer (Scoonhoven, 1968), and lack of mechanical obstruction in or around breeding pools for *Anopheles culicifacies* (Russel and Rao, 1942), and *A. gambi* (Muirhead-Thompson, 1951). Site selection is probably genetically based in all of these invertebrates although experience may be important, particularly in the European corn borer.

Homing to a particular nesting site, territory, or nursery area by fish was reported by Hasler and Wisby (1958), Hasler (1966), Neil (1966), and Lowe-McConnell (1969). From these studies and others it may be concluded that male fish do return to specific breeding areas each year, that during a single breeding season displaced males will return to their nest sites, that territorial males may leave and later return to the same territory, and there is an indication that female mouth brooders return to specific nursery areas. The factors governing the original choice of breeding or nursery site have not been examined completely.

Large-mouthed bass, Micropterus salmoides, selected spawning sites depending upon water depth, cover, and substrate particle size. Hunsaker and Crawford (1964) designed an experimental pond having a water depth of either four feet or eighteen inches or less, cover in the form of large rocks, and four sizes of substrate. The particle size was fine sand, pea gravel (.25 cm.), medium gravel (0.7 cm.), and large gravel (1.5 to 2.0 cm.). The fish chose spawning sites in deep water with cover available and pea size or medium gravel. Cover availability was the most important parameter. The fish were breeding adults. Unfortunately, nothing was reported concerning their previous breeding experience. Two subspecies were used, M. s. salmoides, and M. s. floridana. No difference in spawning-site selection could be detected between these two subspecies because of the type of observations made.

Hagen (1967) examined isolating mechanisms between two forms of the three spined stickleback, Gasterosteus aculeatus. The two forms are trachurus, which is anadromus, and leiurus found in the upper reaches of streams. In laboratory preference tests with wild-caught fish he found distinct preferences for nest-site substrate, nest-site vegetation type, and water velocity during behaviors other than nest building. Trachurus built nests on a sand substrate in the presence of Elodea and in water without a current. In the current preference test, trachurus spent most of its nonnest-building time in the current. In contrast, leiurus built nests over mud in the presence of Oenanthe with no current. These preferences are in good agreement with the distribution of nesting sites of the two forms found in the study stream. Hagen believes that these habitat preferences are under genetic control. Hybrids between the two forms choose intermediate habitats and are narrowly distributed between the two forms. Hagen, therefore, concluded that habitat selection of breeding sites in Gasterosterus is an isolating mechanism which could be reinforced through natural selection outside of the hybrid zone.

A list of factors influencing nest-site selection by birds was given by Hilden (1965). This includes: 1) landscape; 2) terrain; 3) nest, song, lookout, feeding, and drinking sites; and 4) presence of conspecifics. He emphasized that the summation of factors must be taken into consideration in listing any real or potential factors. This summation would give a species a breadth of nesting sites. Experience also plays a role in nest-site selection in birds. The magnitude of this role has not been well documented. The return of breeding pairs year after year to the same nesting site would appear to support this point.

The tree pipit, Anthus trivialis, breeds in meadowlands having at least one tall tree. The meadow pipit, A. praetensis, breeds in open meadows. The only function of the tree for the tree pipit is a landing site following its aerial courtship song; the meadow pipit lands on the ground. Following the construction of a line of telegraph poles across a meadow previously occupied only by the meadow pipit, tree pipits were observed nesting (Lack, 1933).

The presence of holes or burrows for nesting sites is necessary for many birds (Lack, 1933, 1954, 1966; Van Hartman, 1956). The introduction of nesting boxes will attract breeding pairs of birds to woods from which the species previously had been excluded.

Social and non-social stimuli influencing nesting site selection by gulls, Laridae, were discussed by Klopfer and Hailman (1965). These authors believe that social factors are most important in the colony-site selection. Their basis for this belief is the presence of breeding colonies in one area while a similar area nearby is vacant. This latter area may subsequently be occupied whether the former area is still present or not. Why the first pair of a breeding colony select a particular site, or why subsequent pairs should follow their lead, has not been explained.

There is an interaction of experience and genetics in the nest building of the zebra finch, Poephila gottata. Sargent (1965) investigated the effect of rearing and first nesting experience on nest building. Birds were given a choice of color of nesting material, nest substrate, and nest habitat at first nesting and at renesting. The zebra finch does show a preference for the material color, substrate, and habitat of its rearing nest. Pairs renesting were found to be most influenced by previous nesting experience. A bias towards a species-typical color of nest material was evident. Thus, pairs chose brown fibers for nesting regardless of rearing or first-nesting experience. This is the only work which I have been able to find that has demonstrated an interaction between experience and genetics during the reproductive cycle of a species.

Non-reproductive Behavior

Early experience with a particular substrate is sufficient and necessary to affect resting-site selection in Tilapia mossambica. Quertermus (1972) reared fish over a sand or stone substrate for the first sixty days of life. Fish given this experience significantly chose the rearing substrate when tested at either four or eight months of age. Continuous experience was not significantly different from early experience. Under Quertermus' rearing conditions the fish reached sexual maturity between five and six months of age. Fish given late experience, during the last sixty days prior to testing, showed no effect of rearing conditions when tested at four months of age. Those tested at eight months of age avoided the rearing substrate. All fish were observed over a two-day period and tested individually. No selection was made on the first day. On the second day a significant selection was made. Fish feeding on the second day showed an even stronger selection. This appears to indicate that stress has a negative influence on resting-site selection.

Weins (1970, 1972) demonstrated the effect of early experience upon substrate preference in Rana aurora and R. cascadia tadpoles. The preferences were correlated with the typical habitat of these two species. Rana aurora is found in ponds with emergent vegetation. The vertical stems of the vegetation give a striped appearance to the habitat. The habitat of *R. cascadia* is patchy. These patches consist of low rocks and clumps of vegetation. Tadpoles were given fourteen to seventeen days experience over featureless, checkerboard, or striped substrates. *R. aurora* tadpoles (Weins, 1970) reared over a striped substrate showed a distinct preference for that substrate over the checkerboard substrate. No preference was shown by the tadpoles raised over the other two substrates. *R. cascadia* tadpoles, (Weins, 1972), when raised over the same substrates, showed a different preference. A distinct preference was shown for the checkerboard substrate by those tadpoles raised over that substrate. Those raised over either a featureless or striped substrate showed no preference.

Selection of an artificial habitat by *Peromyscus maniculatus bairdi* and *P. m. gracilis* was studied by Harris (1952). The animals were either wild-caught or first-generation laboratory reared adults. Both subspecies selected the habitat which resembled that in which they are typically found. *P. m. bairdi* is found in grassland and *P. m. gracilis* is found in woodland. Hybrids of the two subspecies selected the artificial grassland over the woods. Thus, it appears that the mechanism for the selection of grassland is dominant over that for woodland. In a follow-up study Wecker (1963) investigated the role of early experience upon habitat selection in *P. m. bairdi*.

Wecker (1963) utilized a testing pen which was laid across the boundary between a woodlot and an old field. Field-caught adults and their offspring chose the field habitat. Early experience in a field, woods, or laboratory habitat did not change the preference for the field habitat. Animals reared in the laboratory for twelve to twenty generations did not exhibit a preference. Laboratory animals reared in the field showed a preference for the field habitat. Wecker concluded that there exists in P. m. bairdi an innate preference for a field habitat which may be reinforced by early experience in that habitat. It is probably correct to assume that selection pressures differ in the field and the laboratory. It would be interesting to follow the variation in response to different habitats in cross breedings between field and laboratory strains. To the best of my knowledge this has not been carried out.

Klopfer (1962, 1963, 1965, and 1969) and Klopfer and Hailman (1965) discussed a series of experiments designed to determine the effect of rearing conditions upon habitat selection in the chipping sparrow, Spizella passerina. These results are confounded by a number of variables, especially small sample size.

Wild-caught adults and laboratory-reared birds given no experience chose a pine foliage over oak in a two-choice situation. At the age of two months, half of the birds reared in the presence of oak foliage preferred oak; the other half preferred pine. After this first test these birds were placed in an outdoor enclosure with oak and dogwood foliage. This enclosure was surrounded by pine. At sexual maturity, twelve months of age, all birds preferred pine when retested. The authors concluded that visual experience with the usually-preferred foliage was sufficient to overcome the effect of the rearing experience. This is not necessarily the case. There was apparently no attempt to rear birds in the presence of oak until sexual maturity, without their being able to see pine. Although Klopfer and Hailman have demonstrated that early experience can influence some birds' preference early in life, they have not demonstrated that the preference for pine at sexual maturity is not a built-in mechanism. It is possible that this pine preference is tied to the reproductive cycle.

Basis for Experimental Design

This experiment was designed to test the effect of rearing conditions upon nesting and resting-site selection in certain fish. The species chosen was the mossambique mouth-brooder, *Tilapia mossambica*, a member of the Family Cichlidae. The basis for the design was the following:

1. Wild-caught fish, birds, and mammals have been shown to select particular environments on the basis of specific cues.
2. Experience has an effect upon habitat selection by fish, amphibians, birds, and mammals.
3. The effect of early experience upon resting-site selection by individual *Tilapia mossambica* was demonstrated by Quertermus (1972).
4. It seemed desirable to demonstrate the effect of rearing conditions upon breeding-site selection in a species which migrates to a specific spawning area. Previous studies have not done this.

The influence of prior experience upon nesting-site selection was an open question.

Tilapia mossambica is a riverine species. Lowe-McConnell (1969) and Fryer and Iles (1972) pointed out the plasticity in response to environmental conditions by riverine species, particularly members of the genus _Tilapia_. However, this species, as well as many others, has specific requirements for breeding sites. Also, in the mouth-brooders the fertilized eggs are removed from the vicinity of the breeding site by the female. Thus, the fry are first exposed to a nursery area which is distinct from the breeding area. The nursery area differs from the breeding area in water depth, vegetation, and probably a number of other physical parameters. It therefore seems probable that breeding-site selection has a genetic basis. It is also possible that the presence of older males on the breeding area has an effect upon the location of breeding sites for males breeding for the first time. This has not been demonstrated. According to Neil (1966) it is the male of the species who selects a breeding site. The female is led to the prepared nest site by the male.

As previously noted, _Tilapia mossambica_ is a maternal brooder (see Figure 1). It is common in the eastward flowing rivers of Africa from the lower Zambesi south to Algoa Bay. The rivers are slow-moving and muddy with a substrate of mud or coarse river sand 2 to 3 mm. in diameter (Jubb, 1967; Lowe-McConnell, 1959). During the breeding season, September to December, males move into shallow water, establish territories, and dig nests. The nest, or mating, station (Fryer and Iles, 1972) is a shallow depression 20 to 30 cm. in diameter. There is some contradiction in the literature concerning the type of substrate in which these nests are usually constructed. Lowe-McConnell

(1959) stated that the nests are usually found in the mud where hippopotamus tracks serve as the initial site of digging. Jubb (1967) indicated that this species prefers coarse river sand and that the males may dig more than one nest. He gave no experimental evidence for this preference.

After breeding, the female leaves the nest area and remains in a specific nursery area until the young are free-swimming (Lowe-McConnell, 1969; Neil, 1966). The nursery areas are in very shallow back waters and are frequently weed-choked. The young fish gradually move into deeper water as they mature. Their age at sexual maturity is also controversial, varying from three months in laboratory stock (Neil, 1964; Thoms, personal observation) to one to two years in the field (Donnelly, 1969). The latter case may indicate that smaller, younger males are not successful in establishing territories during their first year of life.

The nest of Tilapia mossambica can be distinguished from that of other species of the mossambica complex by the presence of "finger marks" upon the inner surface and a higher rim (see Figure 2). These "finger marks" (Fryer and Iles, 1972) are apparently made by mouth movements of the male. Their significance is not known.

At least two other species of Tilapia are sympatric with T. mossambica in Africa. Another maternal mouth brooder, T. plascida, is superficially like T. mossambica and is found in deep quiet pools with sandy bottoms (Jubb, 1967). A substrate brooder, T. sparrmanii, is found primarily in the middle Zambesi River, but has been introduced into the lower stretches of the river and elsewhere as a forage fish for larger species. Unlike _T. mossambica_ this species is smaller, has a banded color pattern, and is stenohaline. The males are monogamous instead of polygamous as are the mouth-brooders. The species nests near rocks or within the vegetation. A recently separated species, _T. mortimeri_, is found in the middle Zambesi River. The extent of the overlap of these two species, _T. mortimeri_ and _T. mossambica_, is not known. Little of the ecology of _T. mortimeri_ has been studied. Whether any of these species compete with _T. mossambica_ for breeding sites or other resources is not known.

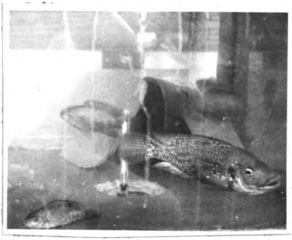


Figure 1. The Mossambique Mouth Brooder, Tilapia mossambica Peters. Note the white chin and sharp-pointed snout and dorsal fin as well as the dark color of the male on the right.

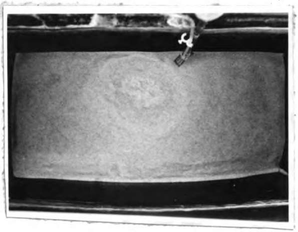


Figure 2. A nest of T. mossambica dug in the sand substrate. This nest is 22.86 cm. in diameter. Note the ridge which is characteristic of a nest of this species. The "finger marks" described by Fryer and Iles (1972) are difficult to see.

MATERIAL AND METHODS

Experimental Animals

The Mossambique mouth brooder, *Tilapia mossambica* Peters, was selected as the species to carry out this research (Figure 1). The line from which the specimens were taken is highly inbred. In 1965 seven animals, four males and three females, were acquired from the American Museum of Natural History, New York. No breeding records were kept on these fish until 1969 when an attempt was made to avoid breeding within a brood. All animals in this laboratory in 1969 were believed to be offspring of a single pair. It is believed that this pair was from the original seven animals. Malformities occur frequently in the broods now being born. No fish having any distinguishable malformity was used in this research. The most common malformities are: crooked jaw, pushed-in faces, swim-bladder disorder, and hazy eyes. In some specimens more than one of these occurred. Those broods having pushed-in faces also had females larger than males and a sex ratio at sexual maturity of four females to one male.

Rearing Conditions

Three rearing substrates were used: fine dark beige sand, natural aquarium gravel (size #4, 2 to 3 mm diameter), and multicolored stone (greatest diameter less than 1.25 cm). The basis for the choice of these was the discussion of breeding sites by Lowe-McConnell (1959) and Jubb (1967). These authors indicated that *Tilapia mossambica* dig nests in either mud or coarse river sand (2 to 3 mm diameter). The fine dark beige sand was a substitute for mud. Attempts to rear adult T. mossambica, or a newly free-swimming brood, over mud failed. The adult fish stirred up the mud so badly that filters could not be run nor could the three 100-mm fish be seen except when they pressed against the glass. The activity of the young fish also stirred up the mud, and most of the fish died in the first three weeks with gills clogged with mud.

The sand was acquired from dune faces along the Lake Michigan shore line between Grand Haven and Holland, Michigan. The #4 natural aquarium gravel closely approximated the coarse river sand in size. It is probably more angular than river material. The gravel used was not new and contained numerous small pieces of charcoal. Prior to use all gravel was passed through one-quarter inch mesh hardware cloth to insure uniformity of particle size. The multicolored stone was washed stream gravel acquired at a local gravel company. Prior to use all stone was passed five times through one-half inch mesh hardware cloth to insure that no piece had a greatest diameter of more than 1.25 cm. The stone was then passed through the same one-quarter inch mesh hardware cloth used for the gravel. This was done to prevent overlap in size ranges. Stone was chosen for two reasons: to provide a larger range of particle size than the species normally encounters in its natural habitat and because pilot studies had shown that males could dig, defend nests, and breed in a stone substrate if no other substrate were available. However, reproductive success was low in this substrate because the eggs were lost in crevices between the stones.

Fish were reared in thirty (30) thirty-eight liter aquaria, 50.5 x 26 x 20 cm. These were glass-covered and separated from adjacent aquaria by black plastic. The water in each aquarium was filtered by either a Le-Bern or Eureka Safety-Flow outside box filter. The filters were filled with charcoal and filter wool. Aquaria were also aerated with an air stone. Four grams of marine salts per gallon were added to tap water run through an activated charcoal filter. The bottoms of the aquaria were covered with 5 cm of substrate. The only other items within the aquaria were filter tubes, and the air stone and its air line.

A water temperature of 26° ± 1° C was maintained by a room air conditioner and heaters. A sixteen-hour on, eight-hour off, photoperiod was used. Lighting was by fluorescent ceiling light or incandescent lights suspended above the aquaria. The lights came on at 0600 hours and went off at 2200 hours. Fish were fed twice daily at approximately 0730 hours and at 1600 hours.

Mouth-brooding females from the breeding tanks were placed into the rearing aquaria while holding eggs or wriggler-stage young. After the young became free-swimming, the females were removed. There were eight breeding tanks. Each was a 75 liter tank containing two males and three females. Females from all eight breeding tanks were used to supply the twenty-four broods used. It is probable, however, that some females were used more than once.

Procedure

This experiment was designed to test the effect of rearing substrate upon nest and resting-site selection. Six experimental groups were used. Early in the data-collection phase it became apparent that there is a difference between resting-site selection (Quertermus, 1972) and nest-site selection. In light of this, a seventh experimental group was added to the design. The experimental design is shown in Table 1. Fish were reared over sand, gravel, or stone substrate. The fish of experimental Groups 1 to 6 were given a choice of the rearing substrate and one of the other substrate. Experimental Group 7 was reared over the stone substrate and tested over the gravel and sand substrates.

All fish were reared over sand, gravel, or stone until sexual maturity, 3.5 to 5.5 months under these rearing conditions. Sexual maturity in males was characterized by black coloration, red color of the fins, white chin, and territory-holding in the rearing aquarium. Sexually mature females were characterized by silver body coloration (Neil, 1964). Color and territory-holding were considered more important than age in selection for maturity. Age at testing was between 5.5 and 9 months. Size at testing was between 65 mm and 120 mm, standard length. Males were larger than females.

In each experimental group, pairs were tested until ten pairs had dug nests in either the rearing or choice substrate. A pair was removed from the experiment when a nest was found. Pairs not digging nests by the end of the eighth day were also removed.

Each experimental group is designated by a number and a letter code. The number of each experimental group is given in Table 1. The letter code is based upon the rearing and choice substrates of each group. The following designations for each substrate are used: sand, Sd; gravel, Gr; stone, St. An example of this designation is as follows: Experimental Group 1, (SdGr). For this group, Sd refers to the rearing sand substrate, while Gr refers to the choice substrate. For all groups the rearing substrate code is given first. Experimental Group 7, which was stone-reared and tested over gravel and sand, is referred to as (StGrSd).

Experimental Tanks

Three 230-liter (120 cm X 56 cm X 34 cm) tanks were used. Each was divided into four equal compartments. Each compartment approximated the size of a rearing aquarium. The dividing barriers were of opaque green plexiglass, which had been sanded to prevent reflection. The inside walls of the tank were lined with nonreflective black plastic. A doorway was made in the dividing barriers by cutting a rectangle out of the center corner, 11 X 5.5 cm (see Figure 3). The bottom of the doorway was 16.5 cm above the surface of the substrate. The depth of the water over the doorway was 4 to 5 cm.

Each of the three experimental tanks contained two of the rearing substrates whose depth was 5 cm. This allowed for all three combinations of the three substrates to be tested during the same time period. In each experimental tank the diagonal compartments contained the same substrate. Each substrate was placed in the opposite diagonal in the two experimental tanks in which it occurred. Thus, the gravel occurred in the front left and back right compartments of Tank #1, and in the front right and back left of Tank #2.

Each compartment had a La-Bern filter filled with charcoal and filter wool. The water was from the same source as that used in

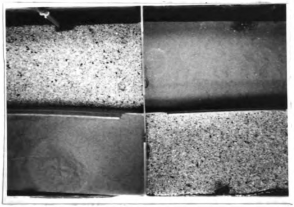


Figure 3. Photograph of Experimental Tank (internal view). This view shows the four compartments of Experimental Tank 2. The substrates are sand and gravel. The tank is divided by opaque green plexiglass barriers. The doorway was made by cutting a 5.5 x 11 cm. rectangle out of the center corner of each barrier. The bottom edge of the doorway is 16.5 cm. above the substrate surface.

Figure 4. Photograph of Experimental Tanks (external view). This view shows the position of the wooden frame around each Experimental Tank. Blue denim cloth was placed over the frame on the top, back, and both sides. The removable curtain across the front of the frame isolated the fish visually from persons walking past. Mirrors were positioned above each Experimental Tank at a 45° angle in order to observe the fish without disturbing them.

the rearing aquaria. Water temperature was maintained at 25° ± 1° C. A wooden frame was built around each experimental tank, and blue denim cloth was placed over the frame on the top, back and both sides (see Figure 4). A removable piece of curtain was placed across the lower half of the front portion of the frame. This extended approximately 12 cm above the top of the experimental tank and served to isolate the fish visually from persons walking past.

Each experimental tank was illuminated by a single 40 watt fluorescent tube suspended 74 cm above the surface of the water. The photoperiod was the same as that for the rearing aquaria. Mirrors were suspended at a 45° angle above the tanks in order to observe the fish without disturbing them. Fish were fed once a day by dropping a pinch of Biorelle at the center doorway. The flake food floated on the surface and spread evenly into all four compartments.

Test Procedures

The fish were tested in pairs. They were released individually from a net at the center doorway at about 1600 hours. After both fish were introduced into the tank, they were chased through the doorway three times with the aid of a net. This gave them experience in crossing the doorway and also involved a visit to both substrates.

Each pair was observed visually for the first time by a volunteer observer approximately sixteen hours after being introduced. This was a position check for both fish. The fish were not marked. It was, therefore, impossible to distinguish males from females by looking down through the mirrors. At approximately 1600 hours (approximately twenty-four hours after introduction and eight hours after the first observation) each experimental tank was checked for the presence of a nest. This was done by systematically passing my hand across the surface of the substrate and feeling for the depression and ridge which characterized a nest (see Figure 2). Pilot studies indicated that this was the most reliable method for detecting a nest.

The presence of a nest was usually impossible to detect by looking through the mirrors or leaning over the tank and looking directly down through the water. If no nest was found, the two daily observations were continued each day until a nest was dug, or until eight days had elapsed. Pilot studies had indicated that pairs not digging in a week were unlikely to dig at all. These non-digging pairs were removed. If a nest was present, the pair was also removed. The greatest diameter and the depth in relation to average depth of smoothed substrate was measured. Each pair was tested once.

Prior to introducing another pair, the substrate was stirred up and then leveled. The water was then allowed to clear and brought up to the appropriate depth.

Statistical Design

The location of the nests dug by each experimental group was analyzed by Chi-Square test. The two dimensions for all nests were analyzed separately. The differences among all six experimental groups were analyzed by one way analysis of variance. The number of days which elapsed before a nest was found was not analyzed. The number of pairs not digging nests in each experimental group was analyzed by

Chi-Square test. The number of fish over the rearing substrate each day was tested by Bonferroni's Chi-Square test (Dayton and Schafer, 1973). The analysis was on pooled data in two categories. Three experimental groups made up each category. The three experimental groups (1, 2, 4) in which the rearing substrate was smaller than the choice substrate were placed in the first category. The other three experimental groups (3, 5, 6) were placed in the second category. Pairs digging nests were analyzed separately from those not digging nests.

The data from the morning observations were converted to the percentage of fish over the rearing substrate for each pair of fish tested. Percentages were used in order not to weight any one pair more than another, since pairs digging nests were removed from the experiment on the day a nest was found. A one way analysis of variance was run to test differences among the experimental groups for all pairs tested. A two way analysis of variance least squares solution for unequal N (Winer, 1962) was used to determine the following:

1. the differences between those experimental groups (Category 1) in which the rearing substrate was smaller than the choice substrate (1, 2, 4), and those (Category 2) in which the rearing substrate was larger than the choice substrate (3, 5, 6) for all pairs tested;

2. the differences between pairs digging and pairs not digging nests for all pairs tested; and

3. the pairs digging nests _versus_ category interaction.

A one way analysis of variance was used to test differences between experimental groups having the same test conditions but different rearing conditions. Percentages of fish over a single substrate were used.

RESULTS

Nesting Site Selection

The number of pairs digging nests in the rearing and choice substrates are shown in Table 1. The number of pairs digging nests in either substrate differed significantly from random for all groups. In experimental groups 1, 3, 5, $p<.05$, while in experimental groups 2, 4, 6, and 7 $p<.01$. In all groups, substrate effects were greater than rearing effects. The fish chose the smallest particle size available, whether or not this was the rearing particle size.

When a sand substrate was available, fish almost invariably dug in sand. There were three exceptions: experimental group 1, pair 13; experimental group 3, pair 12; and experimental group 5, pair 10. In the case of the first pair, which was sand-reared, the nest was dug in gravel on the seventh day after introduction. The pair had been over gravel since the second day following introduction. The other two pairs dug nests in their rearing substrates on the first and second days respectively.

Eight pairs dug two nests, which always were in the same substrate. Five of these pairs were found together over one of the nests. In the other three instances, one of the fish was over a nest while the other was in another compartment with a different substrate and no nest.

Table 1. Influence of rearing substrates on nest-site selection.

Rearing Substrates	Test Conditions	Pairs Digging Nests		Pairs Not Digging
		Rearing	Choice	
Sand	Sand *vs*. Gravel	9	1	4
Sand	Sand *vs*. Stone	10	0	4
Gravel	Gravel *vs*. Sand	1	9	5
Gravel	Gravel *vs*. Stone	10	0	9
Stone	Stone *vs*. Sand	1	9	3
Stone	Stone *vs*. Gravel	0	10	12
Stone*	Gravel *vs*. Sand	0*	10	4

* Gravel is designated as the rearing substrate because of its heterogeneity of color and its particle size.

Table 2. Influence of rearing substrates on nest dimensions.

Rearing Substrates	Test Conditions	Nest Dimensions In Substrates (cm.)					
		Rearing			Choice		
		Diameter	Depth	(N)*	Diameter	Depth	(N)
Sand	Sand *vs*. Gravel	10.41±.69	1.91±.15	10	9.53	1.91	1
Sand	Sand *vs*. Stone	9.30±.41	1.50±.10	11	———	———	——
Gravel	Gravel *vs*. Sand	9.53	1.91	1	9.65±.36	1.80±.13	11
Gravel	Gravel *vs*. Stone	9.09±.43	1.73±.15	10	———	———	——
Stone	Stone *vs*. Sand	8.89	1.00	1	9.80±.25	1.83±.15	9
Stone	Stone *vs*. Gravel	———	———	——	10.44±.48	2.72±.20	11
Stone**	Gravel *vs*. Sand	———	———	——	9.73±.41	1.91±.13	12

* N refers to number of nests.

** Fish tested over substrates other than rearing substrates.

Analyses were made to determine the influence of rearing substrate on nest dimensions. These analyses are made for all nests dug, without regard to substrate. The mean diameters are not significantly different when groups 1 to 6 are analyzed ($F_{.05[5,59]}>1.386$) or when groups 1 to 7 are analyzed ($F_{.05[6,70]}>1.162$).

The mean depths of nests were significantly different among the experimental groups. In the analysis of groups 1 to 6, $F_{.05[5,59]}$ <7.146, while with the addition of experimental group 7, $F_{.05[6,70]}$ <6.146. The differences among the mean depths of nests is accounted for by the nests of experimental group 6 (StGr).

The mean number of days before a nest was found for each experimental group is shown in Table 3. The range of means for all groups was 3.5 to 4.7 days, excluding the single pairs which dug in different substrates from the other nine pairs in experimental groups 1, 3, and 5. Nests were dug on all days.

Resting Site Selection

On nine occasions, a nest was found in a compartment in which no fish had been observed that morning. Six of the nine pairs were observed over their rearing substrate in the morning, and a nest was found in the choice substrate in the afternoon. Five of the six nests were dug in the first three days following introduction. The sixth pair dug a nest on the seventh day. One fish of this pair swam into the compartment later found to contain a nest during the morning observation.

Table 3. Influence of rearing substrates on elapsed time prior to digging nests.

Rearing Substrates	Test Conditions	Days Prior to Digging Nests in Substrates*	
		Rearing	Choice
Sand	Sand _vs_. Gravel	4.0 (1-7)	7.0
Sand	Sand _vs_. Stone	4.2 (2-7)	___
Gravel	Gravel _vs_. Sand	1.0	4.7 (1-8)
Gravel	Gravel _vs_. Stone	4.5 (1-8)	___
Stone	Stone _vs_. Sand	2.0	4.3 (1-7)
Stone	Stone _vs_. Gravel	___	4.6 (1-8)
Stone**	Gravel _vs_. Sand	___	3.5 (1-7)

* All values are expressed as means with ranges in parentheses. Where ranges are not given, only one nest was dug under those conditions.

** Fish tested over substrates other than rearing substrates.

In the seventh pair of these nine, a nest was found in the rearing substrate when the pair had been observed over the choice substrate.

On two occasions fish were observed over the same substrate in which a nest was found but in the other compartment. Once a fish was observed to swim into the compartment containing the nest during the short observation period.

The number of fish not digging in each experimental group is shown in the last column of Table 1. The number of pairs in experimental groups 1 to 6 does not differ from the expected value. The expected value was the mean number of pairs not digging nests for the experimental groups. Four of the experimental groups had three to five pairs not digging nests, while two experimental groups (4 and 6) had nine and twelve pairs not digging, respectively. Sand was present as either the rearing or choice substrate in all four of the experimental groups. Experimental groups 4 and 6 were tested over gravel and stone. Experimental group 7 (StGrSd) had four pairs not digging nests. Sand was also present in this group.

The percentage of fish occurring over the rearing substrate each day is presented graphically in Figures 5, 6, and 7. In trying to analyze the data by days two problems arise. The first is the decreasing number of pairs represented with increasing number of days. Secondly, the number of pairs not digging nests is small.

Because of these problems, experimental groups 1 to 6 were placed into two categories based upon the relationship between experimental rearing-substrate particle size and choice-substrate particle size. In category one, the rearing substrate was smaller than the choice substrate; in category two, the rearing substrate was larger than the choice substrate. Initially, for purposes of analysis, the pairs digging, and the pairs not digging, nests were grouped separately on a daily basis. Subsequently, the performance data for these fish were combined for the entire test period of eight days.

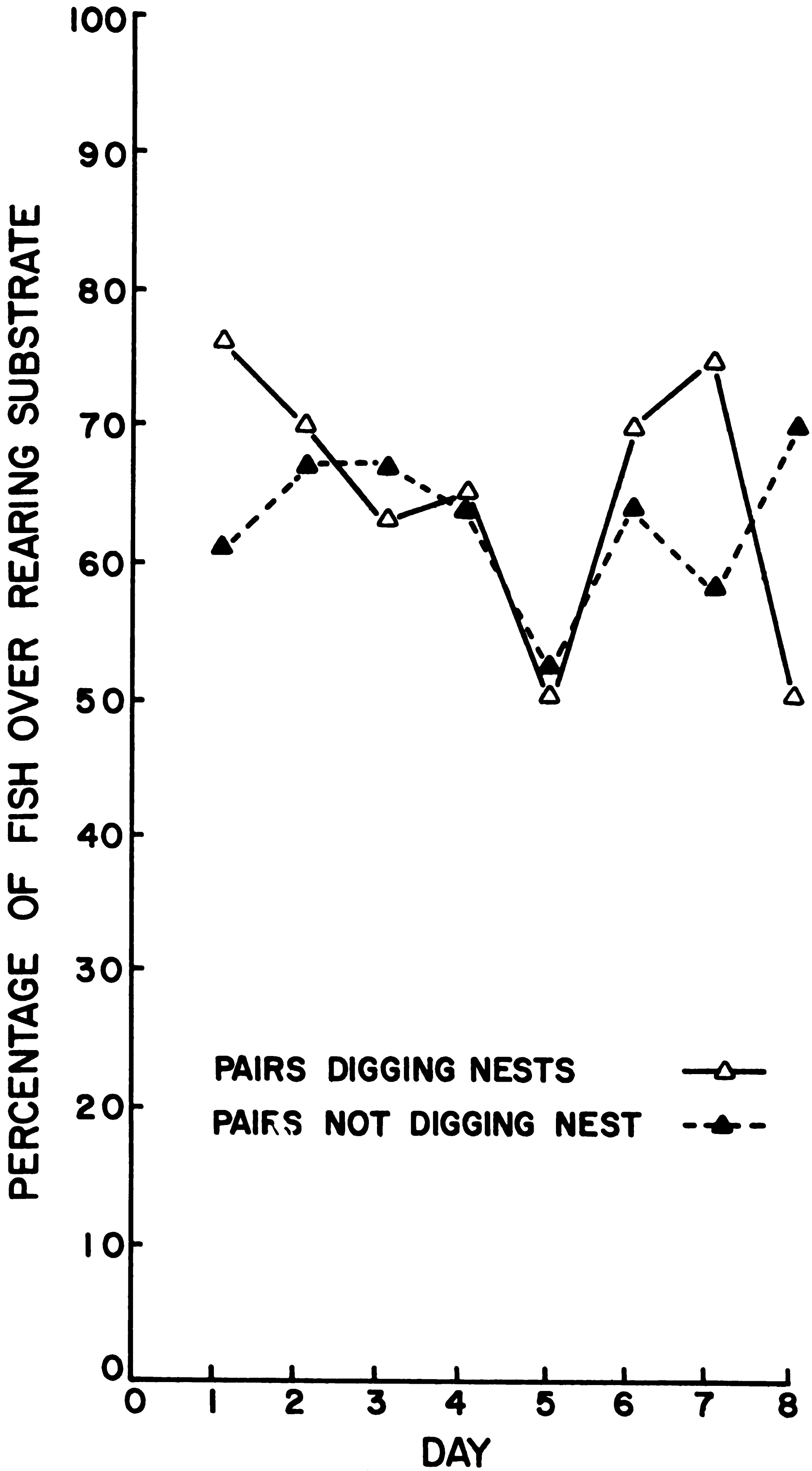
The pairs digging nests in category one (Figure 5) were found significantly more often over the rearing substrate only on days one and two (day one - $\underline{p}<.01$ and day two - $\underline{p}<.05$). Pairs not digging nests did not differ from random on any day. Pairs digging nests and pairs not digging nests did not differ from random in either category two or experimental group 7. It should be remembered that the number of pairs declines across days. This means that a significant deviation from chance is increasingly more difficult to detect.

Figure 5 shows the percentage of fish over the rearing substrate for category one. For the pairs digging nests, the highest percentage (76%) of fish over the rearing substrate occurred on the first day. In contrast, the percentage for non-digging fish was highest on day eight (70%) closely followed by days two and three (67%).

The next figure (Figure 6) represents the percentages for category two. Fish digging nests had the highest percentage over rearing substrate on day six (60%); slightly lower percentages were found on day five (57%) and day one (55%). In comparison, non-digging pairs were concentrated at day three where the percentage was 60%. Only slightly lesser concentrations were noted on day one (57%) and day eight (55%).

Figure 5. Percentage of Fish over Rearing Substrate, Category 1. (Rearing Substrate Smaller than Choice Substrate.) The daily percentages for the pairs digging, and the pairs not digging, nests are shown separately. The number of pairs represented each day for those pairs digging nests is shown in the chart below. The curve for the non-digging pairs represents data from seventeen pairs.

Day Number	1	2	3	4	5	6	7	8
Number of Pairs	30	25	21	16	13	12	6	2



100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE OF FISH OVER REARING SUBSTRATE
PAIRS DIGGING NESTS
PAIRS NOT DIGGING NEST
0
1
2
3
4
5
6
7
8
DAY

Figure 6. Percentage of Fish over Rearing Substrate, Category 2. (Rearing Substrate Larger than Choice Substrate.) The daily percentages for the pairs digging, and the pairs not digging, nests are shown separately. The number of pairs represented each day for those pairs digging nests is shown in the chart below. The curve for the non-digging pairs represents data from twenty pairs.

Day Number	1	2	3	4	5	6	7	8
Number of Pairs	30	24	20	18	15	10	8	4

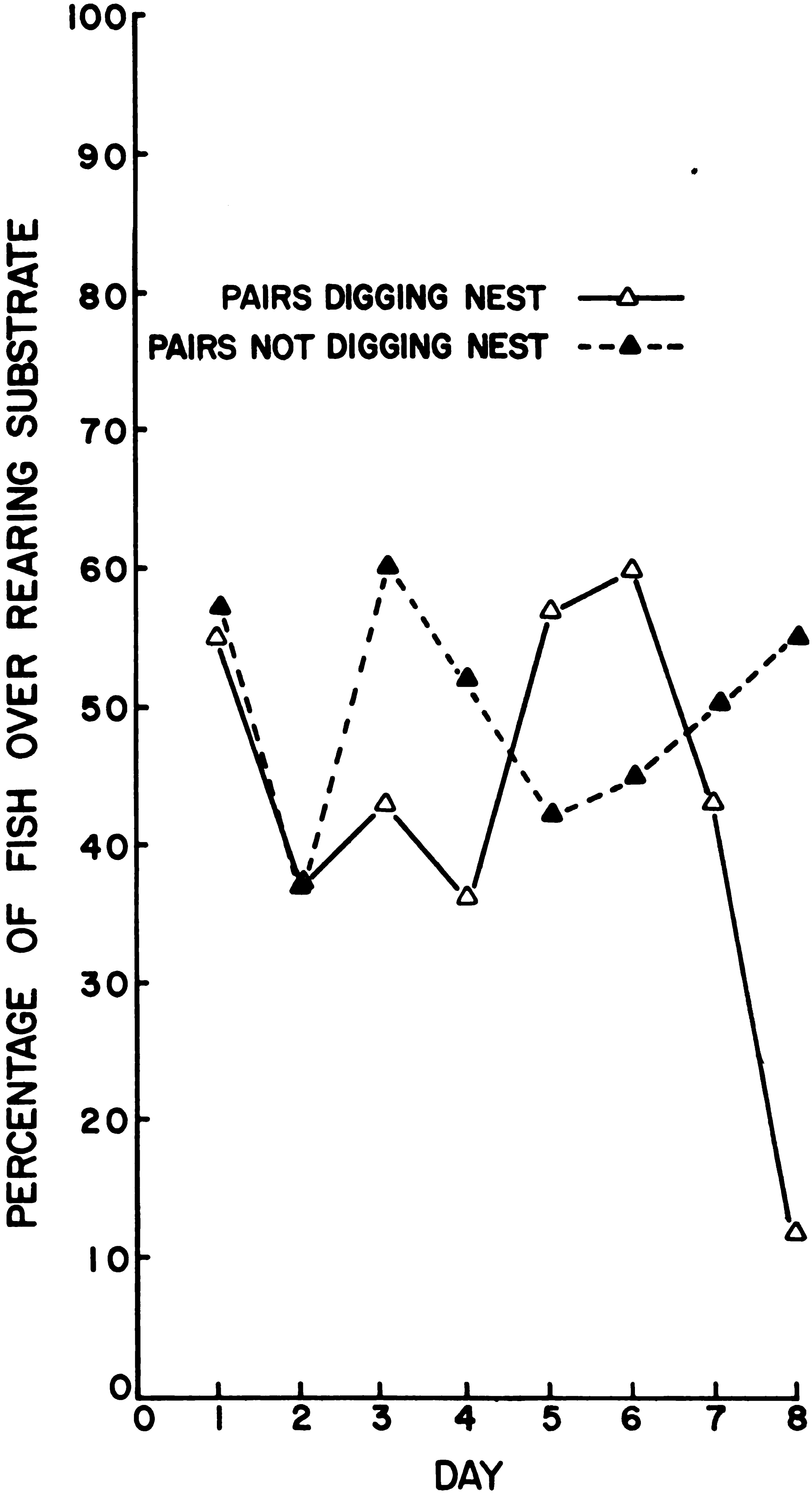
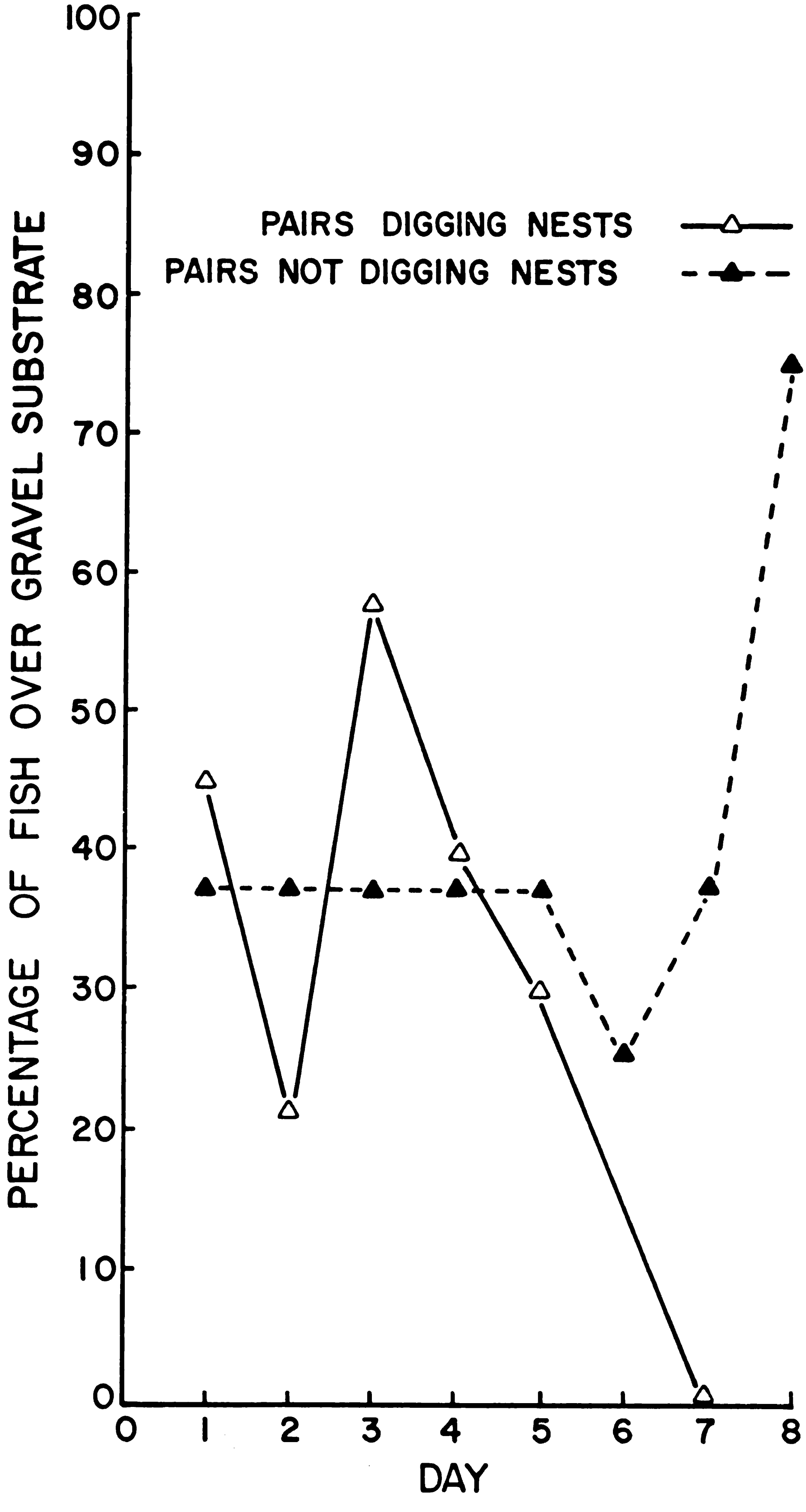

PERCENTAGE OF FISH OVER REARING SUBSTRATE
100
90
80
70
60
50
40
30
20
10
0
PAIRS DIGGING NEST
PAIRS NOT DIGGING NEST
0
1
2
3
4
5
6
7
8
DAY

Figure 7. Percentage of Fish over Gravel Substrate, Experimental Group 7. (Stone-reared; Tested Gravel vs. Sand.) The daily percentages for the pairs digging, and not digging, nests are shown separately. The number of pairs represented each day for those pairs digging nests is shown in the chart below. The curve for the non-digging pairs represents data from four pairs.

Day Number	1	2	3	4	5	6	7	8
Number of Pairs	10	7	6	5	3	3	1	0


PAIRS DIGGING NESTS
PAIRS NOT DIGGING NESTS
PERCENTAGE OF FISH OVER GRAVEL SUBSTRATE
DAY
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8

In experimental group 7 (Figure 7) 58% of the fish in those pairs digging nests were found over the gravel substrate on day three. The second highest percentage was on the first day (45%). The percentage was constant at 37% for the non-digging fish until day six when it dropped to 25%. Day eight was the highest point at 75%.

In category one, of those pairs digging nests more were observed over the rearing substrate than over the choice substrate on six of the observation days. There were two days where the numbers were equal. The fish of those pairs not digging nests occurred more frequently over the rearing substrate on all eight days.

In category two, pairs digging nests occurred over the rearing substrate more frequently on three days. There were five days on which more fish of the non-digging pairs were observed over the rearing substrate. On one day there were equal numbers in each substrate.

In experimental group 7, the pairs digging, and the pairs not digging, nests each had one day in which more fish occurred over the gravel substrate.

The remainder of the statistical tests were based upon combined data from the morning observations. These combined data are presented as the mean percentage of fish over the rearing substrate for each experimental group, as shown in Table 4.

There was a significant difference among the means of experimental groups 1 to 6 ($F_{.01[5,91]}^{<5.48}$). When all seven groups are considered the analysis showed $F_{.01[6,104]}^{<5.153}$. These analyses were

Table 4. Influence of rearing substrates upon fish location at morning observation.

Rearing Conditions	Test Conditions	Mean Percentage of Fish over Rearing Substrates: Pairs Digging Nests	N	Pairs Not Digging Nests	N	All Pairs Combined	N
*1. Sand	Sand vs. Gravel	78.95 ± 8.81	10	71.88 ± 4.03	4	76.93 ± 6.34	14
*2. Sand	Sand vs. Stone	81.84 ± 4.42	10	84.38 ± 5.98	4	82.57 ± 4.69	14
**3. Gravel	Gravel vs. Sand	48.34 ± 10.26	10	53.75 ± 9.19	5	50.14 ± 7.32	15
*4. Gravel	Gravel vs. Stone	61.25 ± 10.48	10	50.69 ± 11.09	9	56.25 ± 7.51	19

**5. Stone	Stone vs. Sand	47.86 ± 9.59	10	60.42 ± 12.67	3	50.76 ± 7.84	13
**6. Stone	Stone vs. Gravel	37.55 ± 9.97	10	46.88 ± 7.07	12	42.64 ± 5.89	22
*Category 1		74.01 ± 5.13	30	63.60 ± 6.91	17	70.25 ± 4.14	47
**Category 2		44.58 ± 5.61	30	50.63 ± 5.07	20	47.00 ± 3.92	50
All Pairs Combined		59.30 ± 4.23	50	56.59 ± 4.27	37	58.26 ± 3.07	97
***7. Stone	Gravel vs. Sand	40.47 ± 11.66	10	40.63 ± 11.55	4	40.52 ± 8.72	14

* Category 1 includes Experimental Groups 1, 2, and 4.

** Category 2 includes Experimental Groups 3, 5, and 6.

*** Fish tested over other than rearing substrate.

All values are given as means and standard error.

N represents the number of pairs of fish.

based both upon the data from the pairs digging and the pairs not digging nests (see Table 4).

The mean percentage of fish in category 1 differed significantly from that in category 2 ($F_{.001[1,93]}>16.541$). The percentages in category 1 were higher than in category 2. This analysis appears to affirm the apparent differences between categories 1 and 2 as shown in Figures 5 and 6.

The pairs digging nests did not differ significantly from the pairs not digging nests ($F_{.05[1,93]}>0.090$). The combined means for each of these two sets of fish is shown in the next-to-last row of Table 4.

The pairs digging, and the pairs not digging, nests appeared to affect the two categories equally. There was no evidence for a digger versus category interaction ($F_{.05[1,93]}>1.965$). All subsequent tests were performed on data from both the pairs digging and the pairs not digging nests.

The size of the rearing substrate in relation to that of the choice substrate does not completely account for the response of the fish. In category 1 (smaller rearing substrates), the percentage of fish over rearing substrate in experimental group 4 was only 56.25%, while in experimental groups 1 and 2, the percentages were 76.93% and 82.57%, respectively. The fact that the rearing substrate (gravel) was smaller than the choice (stone) did not have the same effect upon behavior as did the presence of a rearing sand substrate. Therefore, the presence of a sand substrate, particularly as a rearing substrate, has a strong effect upon the location of the fish.

Comparisons were made between experimental groups having different rearing conditions but with the same test conditions. The analyses were run using the percentage of fish over sand (experimental groups 1, 3; and 2, 5) and gravel (experimental groups 4, 6). There was a significant difference between experimental groups 1 and 3 ($F_{.01[1,91]} < 7.157$). Similarly, data from groups 2 and 5 were significantly different ($F_{.01[1,91]} < 10.101$). Experimental groups 4 and 6 were not significantly different ($F_{.05[1,91]} > 0.017$).

DISCUSSION

Nesting Site Selection

The results of this study indicate that experience does not affect nest-site selection by *Tilapia mossambica*. However, resting-site selection is influenced by an interaction of nest-site preference and the effects of prior experience.

Selection for a nesting site is very specific for *T. mossambica*. Males chose the smallest particle size substrate available in which to dig a nest. This preference for small particle size is not affected by experience.

The evidence presented in this study supports the statements of Lowe-McConnell (1959) and Jubb (1967) that male *T. mossambica* prefer a small particle size substrate for nest digging. The controversial point between these two authors has not been answered. Whether *T. mossambica* males prefer mud, or 2 to 3 mm diameter gravel will have to await a future test. The distinct preference for a sand substrate demonstrated in this study suggests that mud will be the preferred substrate.

Substrate preferences for a breeding site have also been investigated by Hunsacker and Crawford (1964) and Hagen (1967). Neither of these studies investigated the influence of experience. It is interesting to note that the preference of the largemouth bass *Micropterus salmoides* (Hunsaker and Crawford) is for pea-sized gravel. The introduction of Tilapia spp. into Florida has been pointed out by Lachner, et al. (1970). The introduced Tilapia are replacing the native largemouth bass, Micropterus salmoides. This competition does not seem to be based upon nest-site selection according to the findings of Hunsacker and Crawford and of the present study.

Hagen (1967) proposed the separation of Gasterosteus aculeatus into two species on the basis of ecological separation. Part of this separation was due to different demonstrable preferences for breeding sites. Future work with sympatric species of Tilapia mossambica may demonstrate that nest-site selection by Tilapia is a species isolating mechanism.

It appears possible that the fixation for a particular substrate at time of breeding could act as a species-isolating mechanism. There is no direct evidence at the present time which supports this statement fully. Jubb (1967) indicated that the two Tilapia species sympatric with T. mossambica in the Zambesi River are found in different parts of the cross section of the river. The substrate spawning T. sparrmanii is found in weeded areas in the presence of large rocks. This species breeds in these areas.

The other Tilapia species in the lower Zambesi River is, like T. mossambica, a mouth brooder. Jubb indicated that T. plascida is found in deep quiet pools, with a sandy bottom. The reference to sand is to coarse river sand of 2 to 3 mm in diameter. Jubb does not state where this species breeds.

The range of the closely related and recently separated T. mortimeri may overlap that of T. mossambica. A study of the nest-site preferences of _T. mortimeri_ is in the planning stages. As so little has been published about the ecology of this species, the results of the planned laboratory tests will have to await confirmation from field work.

The lack of effect of experience on the substrate preferred for nesting is similar to the findings of Sargeant (1965). The zebra finch, _Poephila gottata_, prefers a brown fiber with which to construct its nest, regardless of rearing or first nesting experience. _T. mossambica_ prefers sand in which to dig a nest, regardless of experience. The gravel used in this study is certainly an adequate substrate for nest construction, and no detectable difference can be found in success of spawning between pairs breeding over a sand or gravel nest.

The finding of a nest in gravel or stone, when sand was present, was perplexing. The digging of a gravel nest by Pair 13 of Experimental Group 1 (SdGr) can not be explained. The other gravel nest and the stone nest dug in the presence of sand were in the rearing substrate. These two nests were dug on the first and second day, respectively. It is possible that these fish were highly motivated to dig nests and did so in their rearing substrate.

Gravel and stone were apparently adequate substrates for nest construction for these two pairs. If time taken to dig a nest is assumed to be a measure of motivation, then both of these pairs were highly motivated.

Eight pairs of fish each dug two nests. This finding supports the field observations of Jubb (1967) of males defending territories containing more than one nest. The function of more than one nest within a territory is not known. Unlike the Salmonidae which construct more than one redd, the eggs of T. mossambica are removed from the breeding site after spawning. The presence of two nests may act as a super sign stimulus to a female entering the breeding area. In this case, a male holding such a territory would have an adaptive advantage during reproduction. Further investigation of this point appears warranted.

In the rearing aquaria, sand and gravel-reared males defended territories and dug nests. No nests were found in the rearing aquaria having a stone substrate. Stone-reared males did defend territories in the corners of the aquaria. It was expected that the experience of digging in the sand and gravel would influence the dimensions of nests in the experimental tanks.

As was seen in Table 2, the diameter dimensions did not differ among the experimental groups. Due to the deep nests dug by the fish of experimental group 6 (StGr), the depth dimensions differed significantly. Other fish, which had the same rearing or test conditions, did not dig nests as large as those of this group.

The single nest dug in stone by Pair 10 in experimental group 5 (StSd) was approximately the same size as the mean for nests dug by other pairs.

The patterns of behavior involved in nest-digging by Tilapia mossambica were described by Bratt (1972, pers. comm). His observations indicated that male T. mossambica first deepen a depression while in a head-down posture. The nest is subsequently widened with mouth movements and with the ventral surface parallel to the substrate surface.

The effect of this two-stage process of nest digging can be seen in Figure 2. This is a nest which measured 10.16 x 1.90 cm when first found. The fish were removed from the experimental tank one week later when this photograph was taken. The dimensions by then were 22.86 x 4.14 cm. All nests in this study were dug in less than 24 hours. It was sometimes possible to detect a ruffling of the substrate surface on a day where subsequently a nest was found. This was rare.

Experience over a particular substrate did not affect the time period between introduction into the experimental tank and the digging of a nest. This would appear to indicate that all males were similarly motivated by the presence of a female. It would be interesting to study the latency to establish a territory and nest using two males. This would more closely approximate field conditions as discussed by Neil (1966).

Resting-Site Selection

The nine occasions upon which neither fish was observed in a compartment later found to contain a nest at first seem to point to a fault in the short duration of the morning observation. Closer inspection of the data has led me to interpret these occasions differently, although acknowledging that a longer observation period would have been desirable.

The territory size of a breeding male in the field is not known. However, if the average size of a nest is between 20 and 30 cm (Jubb, 1967), then my compartments are quite small. My observations could indicate that these nine pairs of fish were using two compartments as a territory. In the first seven pairs the resting substrate differed from the nest substrate and was the same in the last two pairs. The movement of two males during the observation period would appear to support this idea.

Unpublished observations in this laboratory by Quertermus and Bratt suggest that T. mossambica of either sex will choose a resting site on the boundary between two types of substrate. This is why the doorway was placed near the surface rather than at the level of the substrate.

The number of pairs not digging nests in each experimental group is almost exactly the same with the exception of groups 4 (GrSt) and 6 (StGr). These two groups were tested over the same two substrates, gravel and stone. There are a number of possible explanations for the number of pairs that did not dig nests; most are speculative.

Some indication that acclimation may be an important explanation is the observation that several pairs of those not digging nests did not feed while in the experimental tank. No records were kept on feeding by each pair.

Three of the non-digging pairs were observed for more than eight days. None of these dug during the additional seven days of observation. It is possible, however, that these pairs, or any of the other non-digging pairs, would have dug nests given sufficient time. The similarity between the digging and the non-digging pairs when all observations are combined is pointed out by the non-significance of the analysis of percentage of fish over the rearing substrate.

The number of pairs not digging nests in groups 4 and 6 is much higher than in the other five groups. Since both of these groups were tested over the same two substrates, a similar explanation may account for the high number of pairs not digging nests. Sand is the preferred nest-site substrate. This substrate was not presented to these two groups. The data suggest that in the presence of an adequate, but not preferred, substrate for nest digging, fish are less likely to establish breeding areas. Gravel is an adequate substrate for nest construction and is the substrate used in breeding tanks in this laboratory with good results.

The analysis of the data from the morning observation appears to indicate an interaction between selection of a breeding site and the prior experience with the rearing substrate. This interaction is apparent whether the analysis is performed on a daily basis, or on combined data.

As was seen in Figures 5, 6, and 7, there is considerable variation in each of the curves representing the mean percentage of fish over the rearing substrate each day. The distribution each morning was statistically random except for two days. These two days were in category 1, for the pairs digging nests.

The random distribution of the pairs not digging nests in category 1 was surprising. The percentages each day for the non-digging fish are similar to those of the pairs digging nests, though they are lower. In terms of the total number of days during which more fish occurred over the rearing substrate they are virtually the same. This suggests that the effect of prior experience is important in the behavior of the non-digging pairs.

In category 2, the distribution of the fish is random on all days. On day eight, the pairs that dug nests had the least random distribution. Seven of the eight fish still being observed on this day were in the choice substrate. This indicates that the selection of a substrate suitable for nesting is a more important factor than prior experience with a substrate. This is supported by the fact that more fish, of the pairs digging nests, were found over the rearing substrate on only three of the eight test days.

The effect of prior experience appears to have been more important for the non-digging pairs in category 2. The curves of the pairs digging and the pairs not digging nests are similar, although the curve for the latter is slightly higher. More fish of the pairs not digging were found over the rearing substrate on five days.

The fish of experimental group 7 (StGrSd) had no prior experience with either test substrate. The distribution was random each day. Figure 7 shows the percentage of fish over the gravel substrate. The heterogeneity of color in the gravel and the particle size were thought to make this substrate most like the stone. The fish in this group did not respond as if there were any influence of the rearing stone substrate. The curve for the pairs digging nests fluctuates widely. In contrast, the curve for the pairs not digging nests does not fluctuate except for days six and eight. The fluctuation on day six is accounted for by the movement of a single fish. The two curves are lower than the respective curves for the fish of category 2. The total number of days during which more fish were found over a single substrate (sand) is more like that for the fish in category 1.

The importance of the physiological state of an animal in a study of the role of prior experience was discussed by Klopfer (1965) and Quertermus (1972). The data in the present study are too limited to be more than suggestive. However, it may indicate a differential response to the rearing substrate by those pairs which ultimately dug nests and those which did not. The monitoring technique now being developed should give sufficient data daily for each pair to establish where the suggested differences in behavior occur.

Stress has a negative influence on resting-site selection by individual Tilapia mossambica. Quertermus (1972) found no selection for a resting site on the first day following introduction by four and eight month old T. mossambica. He stated that fish must be acclimated to the test aquarium before a significant selection would be made. Most of the pairs in the present study acclimated rapidly. The presence of two fish in the experimental tank, and the dominant status of the male at the time of testing, probably influenced rapid acclimation.

The rate of acclimation apparently differs with the number of fish being tested at one time, and also between species. Individual wild-caught juvenile manini, Acanthurus triostegus sandvicensis, select a particular habitat very quickly (Sale, 1968, 1969). In these studies, the initial selection was for a habitat having shelter and/or deep water. The acclimation period was sixty-five minutes. A similar rapid selection by small groups of the reef fish Dascyllus aruanus was found by Sale (1971). In his study, wild-caught juveniles selected the species of coral in which they had been found within two to seven hours. Some question as to whether the fish in these studies had actually acclimated to the test situation was raised by Quertermus (1972).

The study made by Quertermus provided a comparison between the sand- and stone-reared fish tested in this study. The fish of both studies had been given continuous experience over sand or stone, were sexually mature, and were given a choice of sand or stone. Quertermus tested fish individually. On day one, my sand reared fish (experimental group 2, SdSt), were found 82.1% of the time over sand (pairs digging nests 75%, pairs not digging 100%). Those of Quertermus were found there 43.5% of the time. A similar difference is seen on day two, 89.2% in the present study as compared to 48.1% in his. Only when the percentages (83.4%) from those fish feeding on the second day in Quertermus' study are used do they approach the percentages from my study. These differences are believed to represent a more rapid acclimation of the fish used in this study as well as a selection for a substrate suitable for nest digging.

The stone-reared fish in the two studies responded more similarly than did the sand-reared fish. My fish selected sand in 35% of the observations on day one. No selection was made by those studied by Quertermus. There is a considerable difference between the pairs digging nests (50.0%) and the pairs not digging nests (0%) in my study. This difference is believed to be due to the selection of a nesting substrate by the pairs digging. Four of the ten pairs had dug nests by the end of the second day.

On day two, the fish were evenly distributed between sand and stone. There was no difference between the pairs digging, and the pairs not digging, nests. On the other hand, Quertermus found that his fish had begun to select the stone substrate. His fish were over sand substrate only 30% of the observation time, while fish feeding on day two were over sand only 16.7% of the time. Fish respond differently to the various combinations of rearing and choice substrates. This differential response was seen in terms of mean percentage of fish over the rearing substrate in Table 4. In an attempt to isolate the causes of the different responses a series of analyses were carried out which combined the data in several ways.

The difference among experimental groups was not influenced by differences between those pairs which dug nests and those which did not. The mean percentages for this comparison were 59.30% and 56.59%, respectively.

There is a difference between categories 1 and 2. The means were 70.25% and 47%, respectively. This difference is similar to that seen in Figures 5 and 6. However, as can be seen in Table 4, the mean percentage for experimental group 4 is 56.25%. This is more similar to the percentages of the experimental groups in category 2 than in category 1.

If the relationship between rearing substrate particle size and choice substrate particle size does not account for the behavior of the fish, then the differences between experimental groups should be based on some other parameter. Prior experience did have an influence on their behavior. This was demonstrated by comparison of experimental groups having the same test, but different rearing, conditions. Experimental groups 1 (SdGr) and 2 (SdSt) were significantly different from groups 3 (GrSd) and 5 (StSd), respectively. As can be seen in Table 4, the mean percentage of fish over the sand substrate for experimental groups 1 and 2 was 76.93% and 82.57%, respectively. In comparison, the means for groups 3 and 5 were 50.14% and 50.76%, respectively. The difference between these figures is believed to represent a measure of the influence of the rearing substrates.

The location of the fish in experimental group 4 (GrSt), and the similarity of this group to group 6 (StGr), is difficult to explain. In experimental group 4, ten of the nineteen pairs were found more often over the gravel substrate. On the other hand, in experimental group 6, also tested over gravel and stone, eleven of the twenty-two pairs were over gravel more frequently. It appears that gravel is not an adequate stimulus (Weins, 1970, 1972) to affect resting-site selection.

Experimental group 7 (StGrSd) was added to this study in order to test the preferences of fish given a choice of two unfamiliar substrates. The choice of testing substrates of gravel and sand presented the fish with an adequate and a preferred substrate. In all the test parameters of nest-site selection the fish of this group behaved like the other fish in the study. The 3.5 day average latency to dig a nest was slightly shorter than those of the other six experimental groups (4.0 to 4.7 days).

Analysis of the data by day suggests that experimental group 7 is most similar to those fish of category 1. The mean percentage data for experimental group 7 are different from those for any other group. It is believed that the influence of nest-site selection is the major factor determining the location of the fish. Comparison of the mean percentages of this group with those of experimental groups 1 and 3, which were also tested over gravel and sand, should indicate the influence of rearing substrate on the behavior of the fish. As can be seen in Table 4, the percentage of fish over sand for experimental group 7 lies between the percentages for groups 1 and 3. This is the expected direction of differences between these groups. This trend suggests that prior experience does influence the location of fish during the reproductive cycle. Further work will be necessary.

Conclusions

This study indicates that nest-site selection is under genetic control. Selection for a nest site is not influenced by experience. Nest diameters are not affected by either prior experience or substrate particle size. Nest depths were significantly different due to the behavior of one group. Also, the elapsed time between introduction to the experimental tank and digging of a nest is not affected by experience.

Resting-site selection by pairs of *T. mossambica* is affected by both selection for a nesting site and prior experience. These two effects act together when the preferred nesting particle size and the rearing substrate particle size are the same. The location of the pairs is random when the particle sizes are different, or when the nesting particle size is adequate but not preferred.

Recommendation for Future Study

There are two major changes that I would make if I repeated this study. First, the fish should be marked so that the positions of individuals could be determined. This is particularly necessary for the location of the males, since, according to Neil (1966) the male *T. mossambica* select a nest site and then lead the female to the prepared nest. The second change would be a continuous monitoring system for fish location. At the present time, a thermistor probe system is being developed. This system will give a 24-hour record of fish location. Position of individuals will still have to be confirmed by observation.

The incorporation of these changes would allow investigation of several of the questions raised by the present study. These include the following: (1) an analysis of the differences in activity patterns and substrate selection between males and females; (2) the effect of prior substrate experience on changes in behavior patterns through time; and (3) a comparison between those pairs that will dig nests and those that will not dig through time.

A series of studies should be initiated to test the nest-site specificity of sympatric species. Affirmative results, as suggested by this study, would have great significance to agencies planning to introduce or control populations of fish species. The introduction of a foreign species to a system with another having similar nesting specificities would lessen the probability of success. Provision of not only adequate, but also preferred, substrates for nesting would increase reproductive success.

A series of field studies should be undertaken to confirm laboratory results. These studies should include not only selection preferences but also studies of variations in preferences among age groups.

SUMMARY

1. The Mossambique mouth brooder, *Tilapia mossambica* (Cichlidae), were reared over either a sand, gravel, or stone substrate. At sexual maturity, five to nine months, pairs of fish were tested in an experimental tank having a choice between the rearing substrate and one of the other substrates. The fish were divided into six experimental groups based upon rearing and choice substrate combinations. Pairs were tested until ten had dug nests in either the rearing or the choice substrate. Those which had not dug nests by the end of the eighth day following introduction were removed.

2. Males chose the smallest particle size available to dig a nest, regardless of prior experience. It is suggested that the specificity for a substrate particle size for nest digging could act as a species-isolating mechanism.

3. Eight males dug two nests in the same substrate. The function of more than one nest in a territory is not known. It may offer a super sign stimulus to a female entering a territory.

4. The diameter dimension of the nests did not differ regardless of experience or the type of substrate. The depths of nest differed significantly. These differences were accounted for by the behavior of one group.

5. Experience over a particular substrate did not affect the time period between introduction and the digging of a nest.

6. Males established territories which in at least nine cases encompassed more than one compartment of the experimental tank. In seven of these nine cases the resting substrate differed from the nesting substrate.

7. The number of pairs which did not dig nests was three to five in the experimental groups in which sand was either the rearing or choice substrate. The two experimental groups which were tested over gravel and stone had nine and twelve pairs of fish not digging nests. The possible explanations for the number of pairs not digging nests is discussed. It is suggested that these fish did not acclimate as rapidly as did those which dug nests. It is also suggested that in the absence of the preferred sand substrate, fish are less likely to breed.

8. There was an interaction between the selection of a substrate suitable for nest digging and the effect of prior experience. This interaction affected the distribution of the fish. The relative importance of nest-site selection and prior experience differed depending on the relationship between rearing-substrate particle size and choice-substrate particle size.

9. A seventh experimental group was added to the original design in order to test the preference of fish given a choice of two unfamiliar substrates (sand-gravel). All nest-site selection parameters of this group were similar to those of the other six groups. The resting site location of the fish differed from that of the other groups. The percentage of fish in this group observed over gravel fell between the percentages of fish over gravel in the two other

groups given the same test conditions. This intermediate percentage is suggestive of the effect of nest-site selection without the effect of prior experience.

10. The data were discussed in relation to previous work on habitat selection. Suggestions for future research are given.

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