PRIOR EXPERIENCE AS A FACTOR IN HABITAT SELECTION OF THE CICHLID FISH TILAPIA MOSSAMBICA

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James C. Braddock
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ABSTRACT

PRIOR EXPERIENCE AS A FACTOR IN HABITAT SELECTION OF THE CICHLID FISH *TILAPIA MOSSAMBICA*

By

Carl John Quertermus, Jr.

An African cichlid fish, *Tilapia mossambica* (Peters) was selected to investigate the role that prior habitat experience may play in determining the choice of a resting habitat in fish. Fish were "conditioned" (reared) to different habitats and later tested for habitat selection in an experimental tank offering a choice of the conditioned and an alternative habitat. Three regimes of experience were used (early, late, and continuous). Early-experience fish received experience with the conditioned habitat the first 60 days of life, late-experience fish the last 60 days before testing, and continuous-experience fish from the time of free-swimming to testing. Within each regime, 20 fish conditioned to a habitat with a sand substrate and 20 conditioned to a habitat with a stone substrate were tested. Half of the fish were tested at four months and half at eight months. Each individual was observed for one hour during a two-day period in the experimental tank. This tank offered a choice of two sand and two stone habitats.

Early and continuous-experience fish (both four and eight months) significantly selected the conditioned habitat on the second day in the testing tank in terms of total time and number of habitat visits. There was no significant difference between fish given early and continuous experience. There was an age difference for late-experience fish on day two. Four-month fish showed no significant effect of prior experience, but eight-month fish strongly avoided the conditioned habitat on day two. Possible explanations for this avoidance are discussed. It is concluded that early experience (before sexual maturity) is not only a sufficient, but also a necessary, condition for habitat fixation in *Tilapia mossambica* as judged by a short term selection of two days. These results are discussed in relation to the natural history of *T. mossambica* and the possible application of this study to fisheries management.

The relationship of stress and habitat selection is discussed in terms of the findings that: (1) selection was made on day two but not on day one; (2) fish that fed on day two had a relatively strong selection for the conditioned habitat; and (3) color patterns indicating fright were relatively more common for fish visiting the unconditioned habitat.

Several behavioral comparisons were made using swim time, number of swim bouts, number of pivots, and number of substrate manipulations. There were no definite differences in activity scores for the comparisons of (1) day one vs. day two and (2) fish in conditioned habitat vs. fish in unconditioned habitat. Stone-conditioned fish did more swimming (time and bouts) and more substrate manipulating than sand-conditioned fish. However, fish (both sand and stone-conditioned) visiting the sand habitat did more swimming (time and bouts) and more substrate manipulating than fish in the stone habitat. Likewise, four-month fish did more swimming (time and bouts) and more substrate manipulating than eight-month fish. Pivoting scores were similar or identical for all comparisons, which suggests that this behavior is unaffected by experience, age, or habitat. Pivoting may be necessary for a fish to maintain its "bearings" or awareness. The results of the behavioral comparisons are discussed in relation to other studies.

PRIOR EXPERIENCE AS A FACTOR IN HABITAT SELECTION OF THE CICHLID FISH *TILAPIA MOSSAMBICA*

By

Carl John Quertermus, Jr.

A THESIS

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[illegible]

To my daughters, Kristin and Lisa

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INTRODUCTION

Naturalists have long recognized that each animal species occupies a particular habitat or is associated with particular habitat parameters. Correlation of various habitat features and the distribution of fish species has received considerable attention (e.g. Forbes, 1907; Hallam, 1959; Norris, 1963; Munther, 1970; Everest, 1969; Braasch and Smith, 1965; Jenkins, 1969; and Tyler, 1971), but only a few studies have been thorough or have considered a whole community of species (e.g. Day and Pearcy, 1968; Welcomme, 1964; and Russell, 1969). Day and Pearcy (1968) studied the benthic species on the continental shelf and slope off Oregon and made correlations of species complexes with water depth and sediment composition. Welcomme (1964) correlated the distribution of the young of various Lake Victoria Tilapia with water temperature, depth, dissolved oxygen, and distance from shore. Russell (1969) did a correlation analysis of various physical features of the habitat and the distribution of 62 marine, rocky, shore species of New Zealand.

These correlation studies suggest that any one species utilizes only certain habitats. Therefore, fish must discriminate between habitats and select among the variety available to them. Correlation, however, does not necessarily imply selection (Klopfer, 1969). In order to determine whether fish are actually selecting a habitat it is necessary to test them under controlled conditions where a variety of habitats are available. If a species spends significantly more time in one of the available habitats, a selection is being made.

In recent years various species of fish have been tested to determine their habitat preferences. Baker (1971) observed that 94% of the fourspine sticklebacks in a fresh-water stream were restricted to a habitat comprising about 1% of the total stream study area. The distinguishing characteristic of this habitat appeared to be the presence of *Elodea canadensis*. In a choice situation in the laboratory the sticklebacks selected an *Elodea* habitat over a *Potamogeton* habitat, which was also available in the field. In a more intensive study, Sale (1968 and 1969b) showed that juvenile manini, *Acanthurus triostegus*, select a habitat primarily on the presence of cover, substrate, algal food, and suitable water depth. The presence of conspecifics and water movement are of minor importance, and the following are of slight or no importance: light intensity, substrate color and texture, temperature, salinity, oxygen content, and distance from shore. Several other studies have dealt with various aspects of habitat selection in various species of *Salmo* and *Micropterus* (Baldes and Vincent, 1969; McCrimmon and Kwain, 1966; Kwain and McCrimmon, 1967; Haines and Butler, 1969; Butler and Hawthorne, 1968; and Hunsaker and Crawford, 1964).

As with other behavior patterns, considerable controversy has been raised regarding the basis for habitat selection. Is habitat selection genetically determined or is it a learned response? Surprisingly few studies on vertebrates have considered this question. Harris (1952) utilizing the deermouse, *Peromyscus maniculatus*, was one of the first to study this problem. He laboratory-raised two subspecies of *P. maniculatus*. One subspecies is found in a field habitat and the other in a woodland habitat. As adults, both subspecies selected their appropriate habitat without ever being exposed to it earlier in life.

These results suggest a genetic determination for habitat selection in this species, but the possibility that learning might also be involved was not ruled out since it was not tested. Klopfer (1963) also found that habitat selection in Chipping Sparrows is at least partly determined by heredity. Hand-reared Chipping Sparrows selected normally preferred pine foliage.

The possibility that learning (prior experience) may be of importance in habitat selection of vertebrates has only recently been tested. Wecker (1963 and 1964) worked with the prairie deermouse, Peromyscus maniculatus bairdi, and found that habitat selection in this subspecies is determined primarily by heredity, although prior experience with a field habitat can reinforce this "innate" preference. He also found that early (prior) experience in other habitats cannot override the normal affinity of the field stock for a field habitat. A laboratory stock (12-20 generations), however, lost most of the hereditary control and was much more influenced by prior experience with a habitat.

Klopfer (1963, 1965, and 1967) and Klopfer and Hailman (1965) have investigated the importance of prior habitat experience on subsequent habitat selection in Chipping Sparrows and Blue Tanagers. Their results indicate heredity is of most importance, with learning having some effect. For example, field-caught or hand-reared Chipping Sparrows showed a preference for pine foliage over oak, but birds raised in the presence of oak foliage showed a decreased preference for pine. However, by four to six months the decreased preference disappeared. Sargent (1965) found that previous nesting experience influenced subsequent nestings in the Zebra Finch. Only certain aspects of nesting seem to be learned (nesting material and nest location) while others are not apparently influenced by experience (substrate).

A recent investigation (Wiens, 1970) suggests prior experience is of most importance in habitat selection of *Rana aurora* tadpoles. Tadpoles raised in plain tanks (controls) and those raised in tanks with the walls and bottom colored in a checkerboard pattern showed no preference in a choice situation of a checkerboard or a striped patterned habitat. But individuals raised in a tank with black and white-striped walls and bottom had a strong preference for the striped habitat.

With the possible exception of the numerous studies that have verified the importance of olfactory imprinting of a spawning "habitat" in Pacific salmonids (e.g. Hasler, 1966), there have been no studies that have investigated the possible importance that prior experience may play in fish habitat selection. However, there have been several studies suggesting that various habitat features are learned (e.g. Aronson, 1951; Breder, 1950 and 1954; Hasler, 1956 and 1966; Hasler and Wisby, 1958; and Sale, 1971). Aronson's (1951) study on the interridal gobiid fish, *Bathygobius soporator*, indicates that these fish must utilize learned features of the habitat when jumping from one tide pool to another during low tide. Hasler's (1966) findings that white bass populations in Lake Mendota return to the same spawning area year after year suggests learning of one or more features of the habitat. Recently, Sale (1971) observed that two populations of a coral-reef fish, *Dascyllus aruanus*, were associated with two different habitats differing with regard to the species of coral present. In a laboratory choice situation both populations selected the coral type they were found associated with in the field. These field observations and the investigations of fish learning ability by experimental psychologists of the 1960's (e.g. Behrend and Bitterman, 1964; Otis and Cerf, 1963; and Werboff and Lloyd, 1963) have discredited the long established notion that fish behavior is simply a product of their heredity.

With the preceding knowledge: (1) that at least some species of fish do indeed select their habitats; (2) that prior experience is of some importance in habitat selection of other vertebrates; and (3) that fish are capable of learning, I designed an investigation to ascertain the possible importance of prior habitat experience and subsequent selection of a habitat. The experiment was designed not only to question the importance of prior experience, but also to establish when in the life of a fish such experience is necessary to establish a preference for a habitat and at what age this experience is utilized in selecting a habitat. I especially wanted to determine the role that early experience might play. The studies of Wecker (1963) and Klopfer (1963) indicate experience with a habitat early in the life of an animal may be of importance in the later selection of a habitat. Also, investigations with a wide variety of animals have shown that early experience is of most importance in the establishment of their adult behavior patterns and preferences (e.g. Beach and Jaynes, 1954; Newton and Levine, 1968).

In order to complete this investigation in a reasonable period of time, a species of fish was selected that is predictably bred and easily maintained in the laboratory and that matures at an early age. An African cichlid, Tilapia mossambica (Peters), fulfills these requirements and pilot work showed this was a favorable species to use for the study.

NATURAL HISTORY OF TILAPIA MOSSAMBICA

Distribution

Tilapia mossambica (family Cichlidae) is indigenous to the lower reaches of river systems in East and South Africa from the lower Zambesi River south to Algoa Bay (Fryer, pers. comm.; Jubb, 1961; Trewaves, 1966). Copley (1958), however, claims the distribution of the species extends as far north as the Juba River, some 15° north of the Zambesi. At the southern end of its distribution, T. mossambica is restricted to estuaries (Allanson, Bok, and van Wyk, 1971).

T. mossambica has been introduced widely in tropical and subtropical waters. The first introduction outside Africa was to Java in 1939 (Chen, 1953). Since that time, introductions have been made to Malaya, the Philippines, Thailand, West Indies, Taiwan, Japan, India, Formosa, Ceylon, Pakistan, Trinidad, Borneo, Burma, Malacca, Sumatra, Puerto Rico, many South Pacific islands including Hawaii, the United States, and elsewhere (Chen, 1953; Chimitis, 1955; Swingle, 1960; Devambez, 1964). In the United States the species is established in California, Arizona, Florida, Texas, and a warm spring pond in Montana (St. Amant, 1966; Hoover and St. Amant, 1970; Lachner, Robins, and Courtenay, 1970).

T. mossambica has numerous common names (Crass, 1964; Piennar, 1968). In the United States it has been designated the mozambique mouth-brooder (Bailey et al., 1970). Considerable confusion has reigned over the classification of this species, and T. mossambica is now equivalent to T. (Chromis) vorax Pfeffer, T. (Chromis) natalensis Weber, and T. arnoldi Gilchrist and Thompson (Trewaves, 1966). T. mortimeri from the middle Zambesi is very closely related to T. mossambica and the two species have only recently been separated (Trewaves, 1966). A taxonomical description of T. mossambica is found in Trewaves (1966), Crass (1964), Piennar (1968), and Jubb (1961). A classification for the entire Tilapia genus is found in Thys van den Audenaerde (1968).

Ecology and Behavior

In its native African habitat T. mossambica primarily inhabits rivers and estuaries but also occurs in lagoons and swampy lakes (Fryer, pers. comm.; Jubb, pers. comm.; Piennar, 1968). In rivers the species inhabits deep, sluggish pools with considerable marginal vegetation, especially Phragmites and Typha and other aquatic vegetation such as Aponogeton and Potamogeton (Jubb, pers. comm.). In lakes it is sometimes found in water more than 10 m deep (Boltt, pers. comm.).

The fish flourishes at water temperatures between 21 C and 26 C (Jubb, pers. comm.). The lower lethal temperature is reported as 8 to 13 C depending on the authority (Jubb, pers. comm.; Crass, pers. comm.; Boltt, pers. comm.; Piennar, 1968; Allanson et al., 1971). However, in saline water this fish can tolerate temperatures to 5 C (Jubb, pers. comm.). The lower tolerance in saline water apparently explains why T. mossambica is restricted to estuaries at the southern part of its distribution (Allanson et al., 1971). The upper lethal temperature is 38.2 C (Allanson and Noble, 1964).

Laboratory tests indicate a higher temperature preferendum than field studies. Badenhuizen (1967) found a mean temperature preference of 28.5 C (27.0 to 33.5) in the lab as compared to 25 C reported by Jubb (1961) in the field. Boltt (pers. comm.) mentioned that young fish have temperature preferenda of a very high value which decreases as the fish increase in size. He suggests that this enables the young to penetrate into shallow pools with high temperatures (40 C) where they feed. His data indicate that immature individuals inhabit shallower water than sexually mature individuals. This observation might be explained by the difference in temperature preference of immature and mature fish.

T. mossambica is euryhaline, tolerating salinities from 0 to 32% (Jubb, pers. comm.). Crass (pers. comm.) mentioned that fish are found in water with a salinity of 50%, but Jubb (pers. comm.) reported that 45% approaches the lethal limit. This remarkable fish is capable of breeding in sea water (Brock, 1954), and there is a rumor that they are established in the Pacific Ocean off Hawaii (Fryer, pers. comm.)! Jubb (pers. comm.) mentioned that T. mossambica can live in organically polluted water with a dissolved oxygen content of 3 ppm or less.

In native waters, breeding takes place during the spring (September through December). Males move into shallower water - usually 3 to 6 feet (Jubb, 1967); less than 7 m in Lake Sibaya (Boltt, pers. comm.) - where they construct a saucer-like nest a foot or more in diameter (Crass, 1964). Jubb (1967 and pers. comm.) claims that a male digs and defends several adjacent nests, but other authorities suggest only one next is prepared. The nests are normally constructed in sandy substrates, but muddy substrates in both rivers and impoundments have been utilized (Boltt, pers. comm.). Males prefer course sand, 2 to 3 mm diameter (Jubb, pers. comm.), and hippopotamus tracks are often used as nests (Piennar, 1968). The fish nest both in vegetation and below the limits of vegetation (Boltt, pers. comm.).

The nest is made by mouth digging and is defended by the resident male (Baerends and Baerends-Van Roon, 1950; Neil, 1964 and 1966). Males are polygamous, and females move into the male territories and after considerable courtship display, spawning begins (Jubb, pers. comm.).

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Baerends and Baerends-Van Roon (1950) and Neil (1964 and 1966) have described courtship, spawning, and parental behavior of this species in the laboratory and Hawaiian ponds.

A female deposits a few eggs (about 10) in the pit of a male (Baerends and Baerends-Van Roon, 1950) and then immediately picks them up with her mouth. The male swims over the place where the eggs were layed and ejects milt, which the female also takes into her mouth (Baerends and Baerends-Van Roon, 1950 and Neil, 1964 and 1966). Other authorities indicate that the male emits his milt over the eggs before the female picks them up (Chimits, 1955; Crass, 1964; Chen, 1953; Jubb, pers. comm.). After spawning the female moves off the breeding grounds and usually selects a shallow back-water area (Jubb, pers. comm.). She broods the eggs in her mouth. The eggs hatch in about 60 hours at 28 C but remain in the female's mouth for another 5 to 8 days (Chen, 1953). At the end of the larval period the young become free-swimming and leave the mother's mouth for the first time. At this point they are about 8 mm total length. The young remain with the mother for a period of time, and when danger threatens, they return to her mouth. After a few weeks the fry form a small shoal but remain in shallow water protected by aquatic vegetation from large predators (Jubb, pers. comm.). The dominant predators are <u>Hydrocynus</u>, <u>Clarias</u>, crocodiles, otters, and birds (Crass, 1964; Piennar, 1968). In Lake Kariba, Africa, fish up to 10 cm in total length prefer water depths up to 30 cm on a gently sloping shoreline, and fish 11 to 19 cm usually inhabit water 30 to 60 cm (Donnelly, 1969). Fish leave the shallow nursery areas for the adult habitat before they are sexually mature. This movement occurs at 17 to 19 cm at the age of one year [?] (Donnelly, 1969).

In nature fish mature as early as two or three months when about 80 to 90 mm total length (Chen, 1953; Chimits, 1955). Under crowded conditions they mature at an older age when only 60 to 70 mm (Jubb, pers. comm.; Quertermus, pers. observation). The number of eggs spawned by a female depends upon her size. An 80-mm fish produces 100 to 150 eggs as compared to 200 to 250 eggs for one of 110 mm, and large females may spawn over 1000 eggs (Chen, 1953). Under adequate conditions a female breeds every 6 to 7 weeks (Crass, 1964), or four times (Jubb, 1967), during the breeding season.

Studies on the growth rate of _T. mossambica_ indicate different rates of growth for different waters. In Malaya, under ideal conditions, fish attain 15 to 20 cm in six months (Chen, 1953). Under crowded conditions growth rate is greatly reduced. Males are larger than females at any given age. For example, at 14 weeks males were 61 g and females only 24 g (Chimits, 1955). In Africa, large males will attain a weight of 3 kg (Jubb, pers. comm.).

T. mossambica is an omnivore. le Roux (1956) reported that fish under 2 inches showed a marked preference for zooplankton, but that phytoplankton was the main food of the population he studied and was already present to a considerable extent in fish under 2 inches. In the 2 to 2.9-inch fish and 3 to 3.9-inch fish, phytoplankton was the most abundant food, comprising 47% and 77% of the respective stomach contents. Munro (1967) examined the stomach contents of 170 _T. mossambica_ (18.5 to 47.3 cm T.L.) from Lake McIlwaine, Rhodesia. Filamentous algae and diatoms (associated with the filamentous algae) comprised 52.1% of the food contents, while higher plants contributed 31.0%. The remainder was composed of dipteran larvae and zooplankton. Jubb (pers. comm.) stated that T. mossambica feed on the less coarse aquatic vegetation, algae, small crustaceans, aquatic larvae, and worms, and at times large specimens will take small fish. Large males are also cannibalistic (Jubb, pers. comm.).

T. mossambica is well adapted to handle plant material. The mouth is large with thick lips, protrusible upper jaw, and teeth on both jaws. The jaw teeth are adapted for scraping the surface of plants and rocks, and the pharyngeal teeth are adapted for grinding (Kamal Pasha, 1964). The intestine is long, 103 cm in a 13 cm fish, (Kamal Pasha, 1964), and the enzyme system is comparable to that of typical herbivorous species (Nagase, 1964). T. mossambica also feeds by shifting bottom material with its mouth. Gill rakers are reduced to small knob-like projections (Kamal Pasha, 1964), which indicates a lack of filter feeding.

Economic and Scientific Value

T. mossambica is of considerable economic and scientific importance. Several detailed behavioral investigations have been carried out utilizing this species (Baerends and Baerends-Van Roon, 1950; Seitz, 1948; Neil, 1964 and 1966; and Ruwet, 1968), and recently physiological studies have been conducted (Solomon and Allanson, 1968; Clemens and Inslee, 1968; Allanson et al., 1971; Allanson and Noble, 1964; Nagase, 1964; Potts et al., 1967). Their morphological development has been described by Chacko and Krishnamurthi (1954) and Panikkar and Tampi (1954).

T. mossambica is cultured in ponds as a source of protein for humans throughout Asia and many parts of Africa (Chacko and Krishnamurth, 1954; Swingle, 1960). It is the second (behind the carp) most important culture fish in rice paddies where the annual yield often exceeds 1,000 kg per hectare (Schuster, 1955). In Alabama ponds, production was 2,945.7 pounds per acre in 111 days with initial stocking of 4,000 fingerlings (Swingle, 1960). With heavy fertilization and heavy feeding the annual production in Thailand ponds was as high as 6 to 8 tons per acre (Pongsuwana, 1956)! T. mossambica is also an important food source in the areas of its natural distribution where it is primarily captured by use of "thrust baskets" (Tinley, 1964).

Due to the herbivorous nature of T. mossambica there was hope that this species could be used to control aquatic vegetation in southern areas of the United States. But tests at the Fish Farming Experimental Station, Stuttgart, Arkansas resulted in overcrowded populations and unsatisfactory control of weeds (Sills, 1970). In Asia and Africa, the species has been successfully used to control malaria by keeping waters free of filamentous algae, a necessary breeding substrate for Anopholes ludlowi (Hofstede and Botke, 1950). The most unusual use of T. mossambica is its use as a live bait for commercial skipjack tuna fishing in Hawaii (Shomura, 1964).

METHODS AND MATERIALS

Species Selection

Tilapia mossambica was selected for this investigation because it is easily bred and maintained in the laboratory. It becomes sexually mature at an early age, five to six and one-half months under my rearing conditions. Also, pilot work suggested that T. mossambica could be conditioned to utilize different habitats. The wide distribution of this species as a result of introductions, and its use in behavioral and physiological studies were other considerations involved in its selection. The specimens used for this study were at least fifth generation laboratory stock. The stock originated from the American Museum of Natural History, New York about 1965.

Selection of a Habitat Parameter for Manipulation

When studying the behavior or ecology of an animal, it is important to understand that animal's perceptual world or "Merkwelt" (von Uexkull, 1934; Klopfer, 1969). Not all physical, chemical, and biological components of an environment are used by a fish in selecting a habitat (Sale, 1969b). Therefore, it was important that I utilize a relevant habitat component for entrainment. Observation of T. mossambica indicated to me that the substrate is a very meaningful habitat feature for this species. For example, T. mossambica establish and defend territiries on the substrate, manipulate the substrate in nest digging and feeding, spawn on the substrate, and spend considerable time resting on or near the substrate. In order to simplify and control the experiment as much as possible, only the type of substrate was manipulated and all other habitat components were held constant or eliminated. Two different substrates were utilized in this study, fine yellow sand and multicolored stone (particle size 0.8 to 2.5 cm, $\bar{x}$ = 1.48 cm greatest dimension). These two substrates, differing both in particle size and color, were selected to maximize the possibility of establishing and detecting preferences. Pilot work indicated that these two substrates were a good choice.

Habitat Conditioning

Fish were raised as a group in 38-liter aquaria (50.5 cm x 26 cm x 29 cm). These were designated "conditioning tanks". Each had a slate, sand, or stone substrate. The "sand tanks" and stone tanks" had 3 to 4-cm depths of sand or stone. Each was continually filtered and aerated by a LeBern outside filter filled with charcoal and filter wool. The only object in the tanks other than the substrate substance was the filter intake tube.

Tap water run through an activated charcoal filter unit was used in all tanks. Four grams of marine salt were added to each gallon of water. The water temperature was maintained at 25 ± 2 C. A photoperiod of 15 hours light and 9 hours dark was used (on at 700 hours and off at 2200 hours). Fluorescent ceiling lights were used. All tanks were covered with glass tops. Black plastic sheets were used to cover the tank sides to prevent the fish from seeing into adjacent tanks. Fish were fed BiOrell flake food twice daily at approximately 900 and 1600 hours.

The mouth brooding behavior of *T. mossambica* facilitated the isolation of broods in the conditioning tanks. A female with young in her mouth was netted from a breeding tank and placed in the desired conditioning tank. After the young became free-swimming, the female was

removed from the conditioning tank. Eight 75-liter breeding tanks were available. One to three adult males and two to four females were held in each breeding tank. This arrangement of breeding tanks insured a ready supply of "pregnant" females.

Experimental Design

The experiment was designed as a three-way factorial analysis (Figure 1) utilizing age at experience, conditioning substrate, and age at testing as the three factors. Three age-at-experience groups were used. The first group received experience in a sand or stone conditioning tank the first 60 days after becoming free-swimming. These fish were then moved to a slate-bottomed conditioning tank. They remained there until they were placed in an experimental tank to be tested for a habitat preference. The second group received experience with a sand or stone substrate the last 60 days before being placed in an experimental tank. Before this 60-day period they were held in a slate-bottomed tank. The third group of fish received continuous experience, from free-swimming to testing, in a sand or stone tank.

Forty fish were tested in each of the three age-at-experience groups. Twenty were conditioned to the sand substrate and 20 to the stone substrate. Ten of each set of 20 fish were tested for a habitat preference at four months of age, and the other 10 were tested at eight months of age. In other words, 10 fish were tested in each of the 12 experimental groups. The experimental groups are numbered consecutively. For example, group one had early experience in sand and was tested at four months, and group 12 had continuous experience in stone and was tested when eight months old. Four-month fish were 39 mm to 62 mm

Age at Experience	Conditioning Substrate	Age at (months) Testing
Early Experience First 60 Days	Sand	4
		8
	Stone	4
		8
Late Experience Last 60 Days	Sand	4
		8
	Stone	4
		8
Continuous Experience Day one to Testing	Sand	4
		8
	Stone	4
		8

Figure 1. Experimental design.

($\bar{x}$ = 50.8) and eight-month fish were 62 mm to 102 mm ($\bar{x}$ = 72.2) total length. Males were larger than females.

Experimental Testing Tank

Three 230-liter (120 cm x 56 cm x 34 cm) testing tanks were used to determine the habitat preferences of experimental fish (Figures 2 and 3). Each was divided into four equal compartments with opaque, non-reflective, green plexiglass barriers. The barriers extended from the top to the bottom of the tank. Each compartment was roughly the size of a conditioning tank. The walls were lined on the inside surface with non-reflective, black plastic sheets to prevent individuals from seeing out or from seeing their own images.

A small doorway (11 cm x 11 cm) was cut at the intersection of the four compartments in the center of each tank. Each doorway was 18 cm above the substrate. Consequently, fish had to swim over a barrier to pass from one compartment to another. The tanks were filled with water to a level 2 to 3 cm above the level of the bottom edge of the doorway. The depth of water used depended on the size of the fish to be placed in the tank. The shallow water in the doorway was generally sufficient to prevent fish from lying in the doorway.

Two compartments in each tank had a stone substrate (2 cm deep) and two had sand (2 cm deep). Diagonal compartments had the same substrate. The use of two sand and two stone compartments allowed a fish to change compartments without forcing a change of habitat. Each compartment had a LeBern outside filter filled with charcoal and filter wool. The filters were turned off and their tubes removed when a fish was present. The water was of the same source as that used in the conditioning tanks.

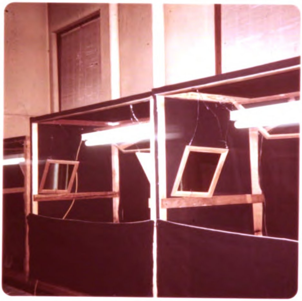

Figure 2. Bank of three experimental tanks.

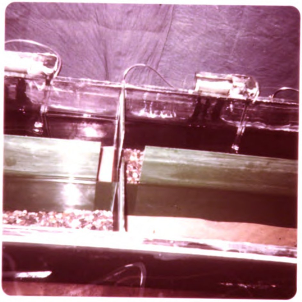

Figure 3. Top view of an experimental tank showing location of doorway and different habitats.

A wooden frame was built around each experimental tank and blue denim cloth was placed on the top, back, and two sides of the frame. A removable denim curtain was placed across the bottom half on the front side. This enclosure reduced outside disturbance and the amount of dust settling into the tanks. A single 40-watt fluorescent tube was suspended 76 cm above the water surface of each tank. The tube extended the length of a tank and was centered (front to back) overhead to prevent shadows being cast by the partitions.

Mirrors were suspended at a 45° angle above the tanks for use in observing the fish. A single mirror, extending the length of a tank, was hung above the back wall and a small mirror was hung on each side. This arrangement permitted observation of all portions of a tank when viewed from a level below the front curtain. The fish could not directly see the observer during an observation session, and none appeared to respond to reflections in the mirrors.

The experimental tanks had the same photoperiod as the conditioning tanks. Their water temperature varied from 21 to 27 C during the course of the investigation. The fish were fed at approximately 900 and 1600 hours by dropping a small portion of BiOrell at the center doorway. The flakes spread evenly into all four compartments. If not eaten, the food remained on the surface for several hours before sinking.

Procedures for Testing

Fish were tested individually in the experimental tanks. Each was placed in an experimental tank at about 1600 hours on the day before the first observation. Each fish was placed in a 600 ml glass beaker, and the beaker was then placed in the central doorway. This condition was maintained for 30 minutes to permit temperature equalization between the water in the beaker and that of the experimental tank. During this time the fish could observe all four compartments. The fish was then randomly dumped into a compartment and, with the aid of a net, was shuttled through the doorway three times. This involved a visit both to a sand and a stone habitat and also provided experience of swimming through the doorway. Pilot work showed the compartment a fish started in had no apparent influence on the fish's subsequent selection. But as a control, in each experimental group, five fish were started in a sand habitat and five in a stone habitat.

Each fish was observed for a total of 60 minutes over a two-day period. The first observation was made approximately 21 hours after introduction (day one). At this time a fish was observed for 15 minutes. A second 15-minute observation was made 30 minutes after completion of the first. On day two, after approximately 45 hours of acclimation, two additional 15-minute observations were made. The procedure was the same as on day one. The first observation on day one and day two was made three to four hours after the morning feeding.

The following procedure was carried out on each experimental tank between consecutive use of fish. Uneaten food was removed. The filters were run for one hour. The water was thoroughly mixed between compartments. The sand surface was leveled and the stone was rolled. Water was added to maintain the proper depth.

During the observation sessions several variables were recorded: total time in each habitat, number of visits to each habitat, and frequency of behavioral measures. Total time in each habitat was measured with a bank of Gra Lab timers. A visit was defined as swimming into a compartment after passing through the doorway. A visit was also scored when a fish swam up into the doorway and then returned to the same compartment. The compartment occupied by a fish at the beginning of an observation was also scored as a visit. Total time and number of visits to each habitat are considered as measures of habitat selection. One or both of these variables were used in the investigations of Klopfer (1963 and 1965), Wecker (1963), Sale (1968, 1969b, and 1971), and Wiens (1970).

The following specific behavior patterns were recorded by hand on a data sheet: swim bouts, total swim time, "pivots", substrate manipulations, and air gulping bouts. These were recorded separately for each habitat. A swim bout was considered to be a period of movement. A period of rest (no movement) of five seconds was used as a criterion to separate adjacent swim bouts. If the interval between two swimming periods was less than five seconds, they were scored as one bout. Total swimming time in each habitat was measured with a stop watch. A "pivot" is a 60° or greater change in position with no forward movement. Substrate manipulations entail picking up a mouthful of sand and spitting it out or mouth-scraping a stone. Air gulping occurs when a fish swims to the surface and mouths air. This was recorded in bouts. Five seconds was used as a criterion to separate adjacent bouts. The above behavior patterns were measured in an attempt to correlate behavior and habitat selection to test Sale's (1969a) model of habitat selection.

In addition to the preceding variables several additional observations were recorded for each fish on each day in the experimental tank. The color pattern was recorded, because Neil (1964) fully described the relation of coloration and behavior in T. mossambica, and color pattern information might be helpful in interpreting my results. During the observation of the first group (group 11), I noticed that some individuals had not eaten. Subsequently, I recorded whether or not a fish had eaten the morning food at the time of the first observation of the day. Mouth-digging in the sand substrate was indicated by the presence of small pit marks on the sand surface. If the sand surface was pitted, I knew the fish had been in those compartments, therefore I recorded the presence or absence of pit marks in both sand compartments.

Statistical Analysis

Three-factor replicated analyses of variance were run on time in the sand habitat and time in the conditioned habitat (Sokal and Rohlf, 1969). Although these two analyses (i.e. time in sand habitat and time in conditioned habitat) are redundant in that they utilize the same values or the complement of the values, they are both necessary to clarify the results. The data fulfilled the assumptions of the analysis of variance without transformation. The analyses were done using time in seconds, but the results are presented in percent time on the tables to facilitate making comparisons between groups. A priori individual degree of freedom comparisons were run on the experience treatment utilizing time in the conditioned habitat. The analysis of visits to different habitats was done by chi-square independence tests. The nature of the behavior results made statistical analysis of behavioral comparisons unnecessary.

RESULTS

Effect of Conditioning

No sex differences were found for eight-month fish; so data for males and females were combined (e.g. for groups 2, 4, 10, and 12, males spent 65.6% and females spent 62.5% of the time in the conditioned habitat). Sex determination of the four-month (immature) fish was not attempted since no sex differences were found in the data for eight-month fish.

Table 1 gives the average percent time spent in the sand habitat. To determine the effect of conditioning, comparison should be made between fish of the same age and age at experience but conditioned to different habitats (e.g. groups one and three or two and four). A difference of zero or near zero for a group comparison indicates no effect of conditioning. On day one there was no effect of conditioning for early or continuous-experience groups. For the late-experience fish a positive effect of conditioning is suggested for four-month fish on day one, but for eight-month fish the difference does not show a positive conditioning effect. Naive fish (i.e. no prior experience with sand or stone) spent 53.3% of the time in the sand habitat on day one (N=30 fish).

On day two the effect of prior experience on habitat selection is apparent. All group comparisons show positive conditioning for the early-experience and continuous-experience groups. The sand-conditioned groups (1, 9, and 10) are close to random (50%) suggesting that only the stone-conditioned fish were positively conditioned. However, tests of naive fish resulted in their spending only 30% of the time in a sand habitat on day two (N=30 fish); therefore, a value of 50% for sand

Table 1. Average percent time spent in the sand habitat on day one, day two, and day two F (fish that fed on day two).

AGE AT EXPERIENCE	CONDITIONING HABITAT	AGE AT TESTING	AVERAGE % TIME IN SAND HABITAT			GROUP #
			DAY ONE	DAY TWO	DAY TWO F	
EARLY EXPERIENCE FIRST 60 DAYS	SAND	4	52.6	58.1	67.5	1
		8	40.0	75.0	83.3	2
	STONE	4	52.6	24.8	27.6	3
		8	51.9	35.0	38.9	4
LATE EXPERIENCE LAST 60 DAYS	SAND	4	50.0	45.0	50.0	5
		8	21.6	19.7	28.2	6
	STONE	4	31.0	43.3	26.6	7
		8	37.6	71.5	76.9	8
CONTINUOUS EXPERIENCE DAY ONE TO TESTING	SAND	4	51.7	49.5	49.4	9
		8	48.1	43.5	83.4	10
	STONE	4	50.0	20.0	42.8*	11
		8	50.0	30.0	16.7	12

*Not from same group of fish as used on day one and day two.

conditioned fish on day two does not mean there was no positive conditioning. Conversely, the results from the naive fish suggest that a value below 30% would be needed for stone-conditioned groups before positive conditioning can be assumed.

The results for the late-experience groups differ from the early and continuous-experience groups. The four-month group comparison indicates no effect of prior experience. The eight-month groups show a substantial effect of conditioning, but the effect is negative in that these fish avoided the conditioned habitat. This avoidance was seen for both the sand and stone-conditioned fish. This reversal was unexpected and difficult to believe; so an additional 10 fish were tested in each group. Again the rejection of the conditioned habitat was present. In this case the observed difference between the sand and stone-conditioned groups for time spent in the sand habitat was 32.5% as compared to 51.8% for the original group comparison. The 19.3% difference between the replicates may be due to the fact that the second replicate was done with 10-month fish with four months of late experience.

The analysis of variance (Table 2) for time spent in the sand habitat indicates the major component of variance is in the second-order interaction. Figure 4 is a graphic presentation of this interaction. The graphs for early experience and continuous experience show the positive effect of conditioning in these groups (sloped lines). A strong interaction is seen on the late-experience graph. The horizontal line for the four-month fish reflects the lack of conditioning effect. The crossing of the lines (at a 45° angle) is due to the rejection of the conditioned habitat by the eight-month fish.

Table 2. Analysis of variance table for time spent in the sand habitat on day two.

SOURCE	df	SS	MS	F	
A (Experience)	2	1,077,489	538,744.5	0.82	n.s.
B (Habitat)	1	1,182,862	1,182,862.0	1.80	n.s.
C (Age)	1	312,120	312,120.0	0.48	n.s.
A x B	2	6,698,430	3,349,215.0	5.10	$.005 < p < .01$
A x C	2	303,546	151,773.0	0.23	n.s.
B x C	1	1,064,460	1,064,460.0	1.62	n.s.
A x B x C	2	72,397,858	36,198,929.0	55.14	$p < .001$
Within	108	70,898,622	656,468.7		
Total	119				

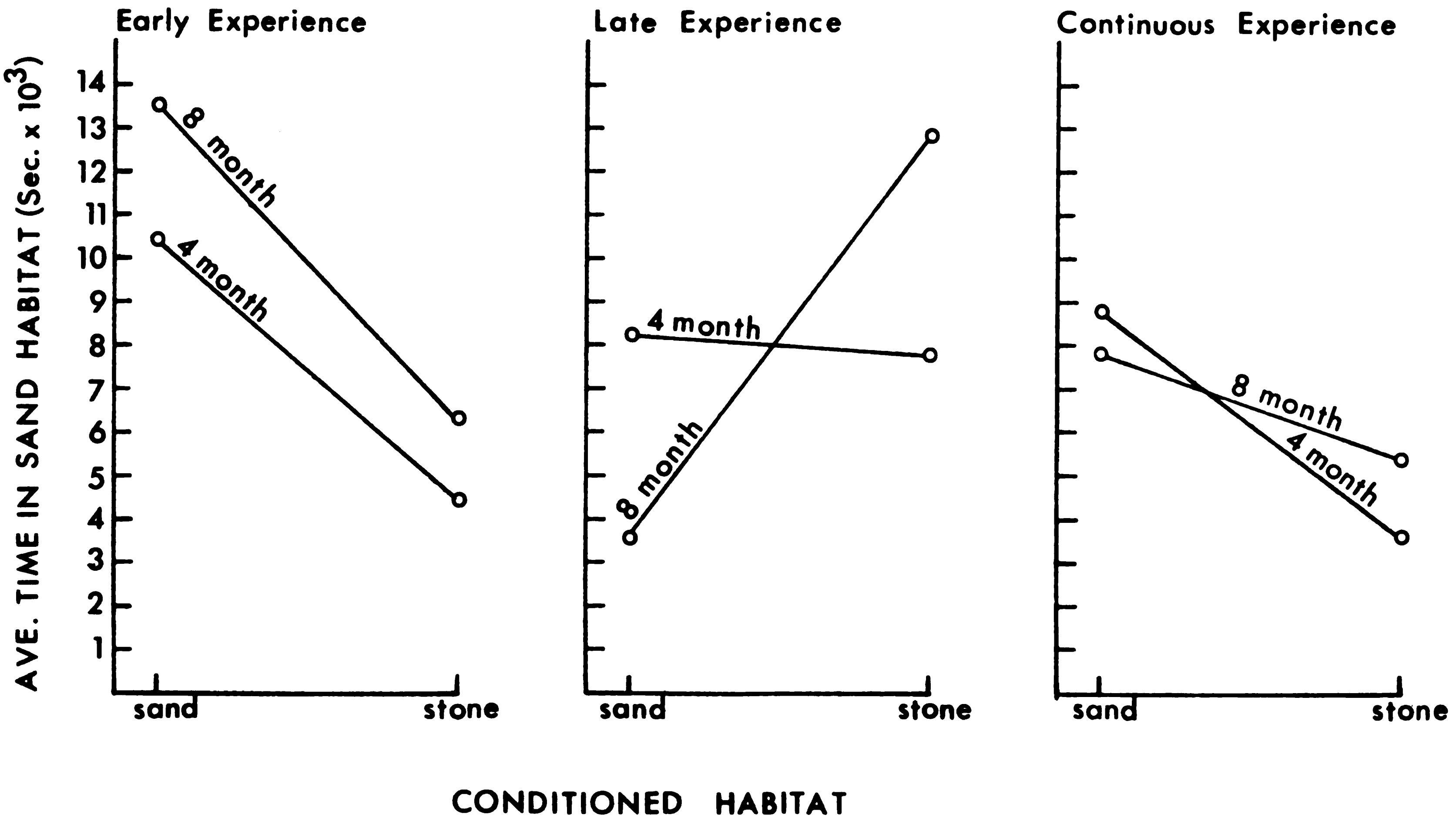

Figure 4. Graphic presentation of second-order interaction for time in the sand habitat.

Day two F (i.e. fish that fed on day two) for the early and continuous-experience groups generally shows a greater positive conditioning effect than that of day two (Table 1). Also, the four month late experience groups show a positive effect of conditioning on day two F, which was not present on day two. The rejection of the conditioned habitat was still strong on day two F for the eight-month late-experience fish. The four-month group with continuous experience in a stone habitat is not the same group as used on day one and day two. This substitute group for day two F was hyperactive compared to other groups, and had a severe fungal infection and subsequent medication during rearing. Therefore, less confidence is placed in this group. None of the other groups experienced any apparent disease during their rearing. Seven eight-month naive fish spent 100% of the time on day two F in a stone habitat.

Table 3 gives the total number of visits to a sand and stone habitat for all fish in each group. These results were analyzed by chi-square two-way independence tests and are summarized in Table 4 (part II). There were no age differences for early and continuous-experience groups (Table 4, part I); so four and eight-month groups were combined in part II. However, there was an age difference for late-experience fish so the analysis was run separately for four and eight-month late-experience fish.

There is no significant effect of prior experience for any group comparison on day one. Early-experience and continuous-experience group comparisons show a significant effect of prior experience on day two. This positive effect is also evident in Table 3. Here seven of eight treatment groups show more visits to the conditioned habitat, and 63.4%

Table 3. Number of visits to sand and stone habitats on day one, day two, and day two F (fish that fed on day two).

AGE AT EXPERIENCE	CONDITIONING HABITAT	AGE AT TESTING	Day One		Day Two		Day Two F	
			visits to sand	visits to stone	visits to sand	visits to stone	visits to sand	visits to stone
EARLY EXPERIENCE FIRST 60 DAYS	SAND	4	12	10	14	9	11	4
		8	8	12	15	5	10	2
	STONE	4	15	14	8	16	8	14
		8	12	12	7	13	7	11
LATE EXPERIENCE LAST 60 DAYS	SAND	4	10	10	9	11	7	7
		8	5	14	5	17	5	11
	STONE	4	15	26	13	16	7	14
		8	9	13	15	7	13	5
CONTINUOUS EXPERIENCE DAY ONE TO TESTING	SAND	4	17	14	13	12	11	10
		8	10	13	10	13	8	3
	STONE	4	10	10	4	16	21*	36*
		8	10	10	6	14	2	10

*Not from same group of fish as used on day one and day two.

Table 4. Analysis of habitat visits by chi-square two-way independence tests.

	chi-square	probability
I. 4 month vs. 8 month fish comparing visits to the conditioned and unconditioned habitat		
A. Early Experience (sand and stone conditioned fish combined)		
Day one (n=40 fish)	0.103	n.s.
Day two (n=40)	0.333	n.s.
Day two F (n=30)	0.045	n.s.
B. Late Experience (sand and stone conditioned fish combined)		
Day one (n=40)	2.253	n.s.
Day two (n=40)	5.467	$.025 > p > .01$
Day two F (n=28)	6.533	$.025 > p > .01$
C. Continuous Experience (sand and stone conditioned fish combined)		
Day one (n=40)	0.386	n.s.
Day two (n=40)	0.685	n.s.
Day two F (n=30)	2.513	n.s.
II. Sand conditioned vs. stone conditioned fish comparing visits to the sand and stone habitats to show effect of conditioning		
A. Early Experience (4 month and 8 month fish combined)		
Day one (n=40)	0.104	n.s.
Day two (n=40)	9.670	$p < .005$
Day two F (n=30)	10.510	$p < .005$
B. Late Experience		
4 month fish:		
Day one (n=20)	0.997	n.s.
Day two (n=20)	0.000	n.s.
Day two F (n=13)	0.972	n.s.
8 month fish:		
Day one (n=20)	0.968	n.s.
Day two (n=20)	9.166	$p < .005$ (reversal)
Day two F (n=15)	5.704	$.025 > p > .01$ (reversal)
C. Continuous Experience (4 month + 8 month fish)		
Day one (n=40)	.000	n.s.
Day two (n=40)	4.889	$.05 > p > .025$
Day two F (n=30)	6.095	$.025 > p > .01$
III. Early experience vs. continuous experience comparing visits to conditioned and unconditioned habitat to show differences in experience treatment		
A. 4 month fish (sand + stone conditioned fish)		
Day one (n=20)	0.038	n.s.
Day two (n=20)	0.004	n.s.
Day two F (n=13)	0.571	n.s.
B. 8 month fish (sand + stone conditioned fish)		
Day one (n=20)	0.011	n.s.
Day two (n=20)	1.461	n.s.
Day two F (n=15)	0.461	n.s.

Table 4. (cont'd)

IV. Early experience vs. late experience comparing visits to conditioned and unconditioned habitats to show differences in experience treatment

A. 4 month fish (sand + stone conditioned fish)		
Day one (n=20)	0.730	n.s.
Day two (n=20)	1.610	n.s.
Day two F (n=13)	0.450	n.s.
B. 8 month fish (sand + stone conditioned fish)		
Day one (n=20)	0.021	n.s.
Day two (n=20)	15.310	$p < .005$
Day two F (n=15)	10.520	$p < .005$

of all visits for these 80 fish were to the conditioned habitat. The chi-square values for the early and continuous-experience groups are greater on day two F than day two. This suggests a greater positive expression of conditioning on day two F than on day two. Furthermore, on day two F (Table 3) all eight groups show more visits to the conditioned habitat, and 66.1% of all visits were made to the conditioned habitat by the 60 fish in the early and continuous-experience groups.

The four-month late-experience groups indicate a slight positive effect of conditioning on day two F (Table 3) but it is not significant. The eight-month late-experience fish show a highly significant ($p < .005$) but negative effect of conditioning on day two. Day two F is also significant ($.025 > p > .01$) but the chi-square value is much lower suggesting that the reversal was not as strong for fish that fed.

Naive fish gave the following results for the number of visits to sand and stone habitats. On day one, 30 of 65 visits were to sand (N=30 fish). On day two, only 15 of 60 visits were to sand (N=30 fish), and on day two F zero of 14 visits were to sand (N=7).

Effect of Age at Experience

Table 5 gives the average percent time in the conditioned habitat for day one, day two, and day two F. The analysis of variance was run on total time on day two and the results are presented in Table 6. The greatest source of variance is in the second-order interaction, which is caused by the reversal of the late-experience eight-month groups. A graph of this interaction would be similar to Figure 4 for the analysis of time in the sand habitat.

Table 5. Average percent time spent in conditioned habitat on day one, day two, and day two F (fish that fed on day two).

AGE AT EXPERIENCE	CONDITIONING HABITAT	AGE AT TESTING	AVERAGE % TIME IN CONDITIONED HABITAT			GROUP #
			DAY ONE	DAY TWO	DAY TWO F	
EARLY EXPERIENCE FIRST 60 DAYS	SAND	4	52.6	58.1	67.5	1
		8	40.0	75.0	83.3	2
	STONE	4	47.4	75.2	72.4	3
		8	48.1	65.0	61.1	4
LATE EXPERIENCE LAST 60 DAYS	SAND	4	50.0	45.0	50.0	5
		8	21.6	19.7	28.2	6
	STONE	4	69.0	56.7	73.4	7
		8	62.4	28.5	23.1	8
CONTINUOUS EXPERIENCE DAY ONE TO TESTING	SAND	4	51.7	49.5	49.4	9
		8	48.1	43.5	83.4	10
	STONE	4	50.0	80.0	57.2*	11
		8	50.0	70.0	83.3	12

*Not from same group of fish as used on day one and day two.

Table 6. Analysis of variance table for time spent in the conditioned habitat on day two.

SOURCE	df	SS	MS	F	
A (Experience)	2	6,698,430	3,349,215.0	5.10	.005 < p < .01
B (Habitat)	1	1,930,403	1,930,403.3	2.94	n.s.
C (Age)	1	1,064,460	1,064,460.0	1.62	n.s.
A x B	2	1,077,489	538,744.5	0.82	n.s.
A x C	2	1,499,237	749,618.6	1.14	n.s.
B x C	1	312,120	312,120.0	0.47	n.s.
A x B x C	2	71,202,168	35,601,083.9	54.23	p < .001
Within	108	70,898,622	656,468.7		
Total	119				

The age-at-experience treatment source of variance is significant on day two (Table 6). Individual degree of freedom comparisons were carried out on the experience treatment (Table 7). There is no significant difference for early as compared to continuous experience. But there is a highly significant ($p < .001$) difference between early or continuous experience as compared to late experience. The late-experience group showed no effect of conditioning for four-month fish and a negative effect of conditioning for the eight-month fish (Table 5).

Table 8 summarizes the time variable for all groups that showed positive conditioning on day two (groups 1-4 and 9-12). All of these eight groups had experience with the sand or stone habitat during the first 60 days of life. On day one the average percent time in the conditioned habitat (48.5%) and the percent of fish (49.3%) that spent more time in the conditioned, as opposed to the unconditioned, habitat were very close to random (50.0%). On day two these two figures (64.5% and 68.8%) reflect the positive effect of conditioning. The increased positive effect on day two F is also apparent (67.1% and 74.1%). The figures for day two F include the substitute group 11 fish. If these were removed from the analysis the difference between day two and day two F would be greater.

Table 4 (parts III and IV) summarizes the statistical analysis for habitat visits in order to show the effect of age at experience. Part III indicates no significant difference between early and continuous experience for both four and eight-month fish on any day. The comparison of early versus late experience (part IV) indicates no significant difference for four-month fish but a highly significant difference ($p < .005$) for eight-month fish on day two and day two F.

Table 7. A priori individual degree of freedom comparisons for experience treatment (factor A) for day two time in conditioned habitat.

Source of Variation	df	SS	MS	F	
Experience	2	6,698,430	3,349,215	5.10	$.005 < p < .01$
Early vs. Continuous	1	369,377	369,377	0.56	n.s.
Early + Continuous vs. Late	1	6,329,053	6,329,053	9.64	$p < .001$
Error	108	70,898,622	656,469		

Table 8. Summary of time variable for all groups with sand or stone experience during the first two months (groups 1-4 + 9-12).

DAY	AVERAGE % TIME IN CONDITIONED HABITAT	% OF FISH THAT SPENT MORE TIME IN CONDITIONED HABITAT THAN UNCONDITIONED HABITAT
DAY ONE (N=80 fish)	48.5	49.3
DAY TWO (N=80 fish)	64.5	68.8
DAY TWO F (N=60 fish)	67.1	74.1

Behavior Analysis

In order to clarify the lack of conditioning effect on habitat selection on day one as compared with day two, I compared the activities on these two days. Table 9 indicates no definite differences in swimming time, number of swim bouts, or number of pivots on day one and two, particularly for groups 1-4 and 9-12, the groups that showed positive conditioning.

Table 10 compares the behavior of fish in the conditioned and unconditioned habitats. The activity levels were corrected for unequal time spent in the two habitats by dividing by the percent time in the corresponding habitat. The eight groups that showed a positive effect of conditioning (groups 1-4 and 9-12) were used in this analysis. When total activities were used, there was a slightly higher activity score for swim bouts, pivots, and substrate manipulations for fish in the familiar (conditioned) habitat as opposed to the unfamiliar habitat. The amount of time swimming was greater in the unfamiliar habitat. However, if the total score for each group is used there was no apparent difference in activity for fish in the familiar and unfamiliar habitat. Thus, an equal number of groups had a higher level of activity in the unfamiliar and familiar habitat.

An analysis was done to determine possible differences in behavior frequencies given by fish while in the sand and stone habitat (Table 11). Frequencies were again corrected for time in each habitat. Swimming time and number of swim bouts were greater in the sand habitat, but in both cases only 7 of 12 groups had greater frequency scores in the sand habitat. The number of pivots per unit time was slightly greater in the sand habitat, but only 5 of 12 groups had more pivots in the sand.

Table 9. Activity comparison of fish on day one and day two. Numbers in parentheses indicate number of fish more active on the indicated day than on the alternate day.

	TOTAL SWIM TIME (SECONDS)		TOTAL SWIM BOUTS		TOTAL PIVOTS	
	DAY ONE	DAY TWO	DAY ONE	DAY TWO	DAY ONE	DAY TWO
ALL GROUPS	7450 (45)	5908 (43)	844 (44)	848 (36)	1195 (58)	1063 (49)
GROUPS 1-4 + 9-12	3692 (28)	4023 (30)	505 (27)	580 (27)	811 (36)	721 (38)

Table 10. Behavior comparison of fish in familiar and unfamiliar habitats for groups 1-4 and 9-12 (day one and day two).

	TOTAL ACTIVITY ÷ % TIME IN FAMILIAR HABITAT	TOTAL ACTIVITY ÷ % TIME IN UNFAMILIAR HABITAT	# OF GROUPS WITH GREATER ACTIVITY PER UNIT TIME IN FAMILIAR HABITAT	# OF GROUPS WITH GREATER ACTIVITY PER UNIT TIME IN UNFAMILIAR HABITAT
SWIM TIME	74.66	84.94	4	4
SWIM BOUTS	11.71	10.49	4	4
PIVOTS	16.12	12.98	5	3
SUBSTRATE MANIPULATION	4.66	3.34	3	5

Table 11. Behavior frequency comparison for behaviors expressed in the sand and stone habitats (all groups).

	TOTAL ACTIVITY ÷ % TIME IN SAND HABITAT	TOTAL ACTIVITY ÷ % TIME IN STONE HABITAT	NUMBER OF GROUPS WITH GREATER ACTIVITY PER UNIT TIME IN SAND HABITAT	NUMBER OF GROUPS WITH GREATER ACTIVITY PER UNIT TIME IN STONE HABITAT
SWIM TIME (SECONDS)	156.95	117.80	7	5
SWIM BOUTS	20.10	14.65	7	5
PIVOTS	23.24	21.56	5	7
SUBSTRATE MANIPULATIONS	6.46	2.51	8	2 (2 ties at zero)

Substrate manipulations were considerably more frequent in the sand habitat as measured by total activity and number of groups. Eight groups did more substrate manipulating in sand and only two did more in stone. Two groups tied at zero.

The preceding analysis suggests that there may be a quantitative difference in behavior patterns observed for fish conditioned to the two different habitats. Table 12 compares the total scores for fish conditioned to the sand and stone habitats. These comparisons were made using all groups for both day one and day two. Stone-conditioned fish spent more time swimming, had a greater number of swim bouts, and did more substrate manipulating. The substantially higher scores for stone conditioned, compared to sand conditioned, fish for these activities is clouded by the fact that only four of the six group comparisons indicate a greater activity for the stone conditioned fish. For all three patterns it is the continuous-experience groups that do not coincide with the early and late-experience groups. Pivoting was the only behavior expressed at a greater frequency by sand-conditioned fish, but only three of six group comparisons show a greater pivoting rate for sand-conditioned fish.

During the course of the observations it was obvious that four-month fish were more active than eight-month fish. An analysis was subsequently done to verify this observation (Table 13). Four-month fish did twice as much swimming and had almost twice as many swim bouts as compared to eight-month fish. Substrate manipulations were also more common for four-month fish (360 compared to 85). The number of pivots, however, was almost identical for four and eight-month fish.

Table 12. Behavior comparison of sand and stone conditioned fish for all groups on day one and day two combined.

	TOTAL ACTIVITY BY FISH CONDITIONED TO SAND	TOTAL ACTIVITY BY FISH CONDITIONED TO STONE	NUMBER OF SAND CONDITIONED VS. STONE CONDITIONED GROUP COMPARISONS WITH GREATER ACTIVITY BY THE STONE CONDITIONED GROUP
SWIM TIME (SEC)	5083	8275	4 of 6
SWIM BOUTS	766	1016	4 of 6
PIVOTS	1248	1011	3 of 6
SUBSTRATE MANIPULATIONS (NUMBER OF 15 MINUTE BINS IN WHICH THE BEHAVIOR OCCURRED)	28	35	4 of 6

Table 13. Behavior frequency comparison of four-month and eight-month fish for all experimental groups combined.

	4 MONTH	8 MONTH	# of 4 vs. 8 MONTH COMPARISONS WITH GREATER ACTIVITY IN 4 MONTH GROUP
SWIM TIME (SEC)	8936	4422	4 of 6
SWIM BOUTS	1108	674	5 of 6
PIVOTS	1108	1151	2 of 6
SUBSTRATE MANIPULATIONS	360 (44)*	85 (19)*	4 of 6 (5 of 6)*
SWIM BOUTS ÷ PIVOTS	1.00	0.59	5 of 6 4 vs. 8 month comparisons show 4 month with relatively more swim bouts than pivots.

* Number of 15 minute observation periods in which the behavior was expressed.

Color Pattern Analysis

The color pattern of each individual was recorded daily at the time of each observation. I analyzed these patterns on the basis of whether or not they occurred in the conditioned or unconditioned habitat (Table 14). A hatched or striped pattern was less common than neutral, barred, or dark for fish in both the conditioned and unconditioned habitat on day one, day two, or day two F. But a hatched or striped pattern was relatively more frequent for fish in an unconditioned, as opposed to those in a conditioned, habitat. This difference is not significant for any day, but approaches significance on day two F ($0.1 > X^2 > .05$). On day two F, 38% of the fish in the unconditioned habitat were hatched or striped whereas only 16% were hatched or striped in the conditioned habitat.

The ability of rapid color change by T. mossambica was evident during this investigation. Individuals changed color intensity within one minute after changing habitats, with fish being much lighter in color in a sand habitat than a stone habitat. However, I did not observe a color pattern change (e.g. barred to hatched) with a change of habitats, although T. mossambica is also capable of a rapid color pattern change (Neil, 1964 and Quertermus, personal observation).

Table 14. Comparison of coloration of fish selecting conditioned and unconditioned habitats for groups 1-4 and 9-12.

	In Conditioned Habitat		In Unconditioned Habitat	
	Number of Fish Hatched or Striped	Number of Fish Neutral, Barred, or Dark	Number of Fish Hatched or Striped	Number of Fish Neutral, Barred, or Dark
Day One	2	32	7	27
Day Two	16	34	8	14
Day Two F	6	31	5	8

DISCUSSION

Importance of Early Experience

The results of this investigation indicate that prior habitat experience may be of primary importance in establishing a preference for a resting habitat in certain fish. Under the conditions of this study, prior habitat experience played a significant role in the habitat selection of *T. mossambica*. However, not all regimes of experience were sufficient to establish a habitat preference as judged on the basis of amount of time or number of visits to a habitat. Fish given experience only late in life (groups five through eight combined) did not select the conditioned habitat, whereas fish given only early experience (first 60 days) or those that received continuous exposure to a habitat selected the conditioned habitat. In other words, early experience was not only a sufficient but also a necessary condition for habitat selection.

The four-month fish given experience late in life (groups five and seven) were positively conditioned to a habitat based on visits for both day two and day two F, but no influence of experience was seen when determined by the time variable except on day two F. This weak positive effect of conditioning (compared to groups 1-4 and 9-12) may have resulted from the fact that although these fish received late experience, this still occurred before they became sexually mature. In other words, for *T. mossambica*, habitat fixation seems to occur before individuals become sexually mature, and the strongest attachment to a habitat is made during the first few weeks of life.

These results seem logical when one considers what is known of the natural history of this species (see Natural History Section). Females carrying young move to shallow water areas with vegetative cover. Here the young are released and are then guarded by the female for a period of time. Fixation to a habitat may well occur during this period. After the young are abandoned by the female, they would remain in this habitat with relative safety from predation. As the young grow, the number of potential predators is reduced allowing movement from the areas of cover. Then, if danger threatens, the response would be to return to a place of safety which was learned earlier in life. There may be other advantages for habitat fixation besides possible avoidance of predation. For example, fixation to a proper habitat may be related to the presence of an adequate food supply. These hypotheses should be tested with detailed field work. In nature, the vegetative component of the habitat may be important in habitat fixation and should be examined.

The present study is one of the first to show that early experience is not only a sufficient but also a necessary condition for determining later behavior. Most investigations of early experience and subsequent behavioral responses have been poorly controlled. The usual procedure has been to give an animal some type of early experience, test at a later time, and then conclude that early experience is important. The control is to give the same experience at other times of life to determine whether the same effect is noted (see King, 1958 for further clarification). Most of the "early experience" studies investigating habitat selection in vertebrates suffer from this lack of control (e.g. Klopfer, 1963 and Wecker, 1963). A notable exception is Wien's (1970) work with tadpoles. He concluded that early experience was sufficient to establish a habitat preference but was not a necessary condition, since tadpoles given the same length of experience later in life gave the same response. Thus, my study is the first to show that early experience is a necessary condition for the establishment of a preference for a resting habitat in a vertebrate.

It is of interest that continuous experience did not cause a stronger fixation to a habitat than early experience. In fact, observations of early-experience fish suggested a greater (but not significant) effect of conditioning than for continuous-experience fish. Early-experience fish averaged 68.3% and continuous-experience fish averaged 60.8% of the time in the conditioned habitat on day two, but the difference between early and continuous experience was not as great on day two F. Also, based on visits there was even less difference on day two between early and continuous-experience groups (i.e. 66.7% and 62.5% of the respective visits were to the conditioned habitat). The fact that continuous experience did not increase habitat fixation over early experience is another indication that only early experience (first two months or less) is important in establishing a habitat preference.

The avoidance of the conditioned habitat by the eight-month late-experience fish is puzzling. The eight-month continuous-experience fish also had immediate prior experience in the conditioned substrate, but they did not reject it. However, the somewhat lower scores for the continuous-experience fish compared to the early-experience fish may be due to a slight avoidance of the conditioned habitat by some individuals.

Two possible explanations may account for the avoidance by the late-experience fish. Since these individuals received early experience on a slate substrate, the avoidance for the habitat experienced late in life may be due to a specific search image (Tinbergen, 1960 and Hinde, 1966) for the "early-conditioned slate habitat".

The second possibility is that the exploration of the novel (unconditioned) habitat was not as complete by the end of the second day for the late-experience fish as it was for the early and continuous-experience fish. Berlyne (1966) and others (see review by Fowler, 1965) have shown that the amount of exploratory behavior shown by an animal (in this case a laboratory rat) depends on the novelty of the test environment. The more novel the environment the more intense and longer the exploration. Perhaps the late-experience fish found the unfamiliar habitat more novel than did early or continuous-experience fish.

Although the avoidance of the conditioned habitat is real on day two, it may not persist indefinitely. Preliminary results of a follow-up study suggest that the avoidance disappears by the fifth or sixth day in the experimental tank, when fish begin to select the conditioned habitat. Ten individuals with late experience on stone spent 65.0% of the time in a stone habitat on days five and six as opposed to only 28.5% on day two. These results are questionable, since seven of the 10 fish were used earlier in the experimental tanks and all 10 were 10 to 11 months old with four to five months experience on stone. This study is continuing with another group of fish. If these results are verified, the second explanation for the avoidance seems the most likely.

Naive fish (no experience with sand or stone), tested for a selection of a sand or stone preference, strongly selected the stone habitat (70.0% on day two, N = 30 four and eight-month fish, and 100% on day two F, N = 7 eight-month fish). There are several possible explanations for these results. *T. mossambica* may have an "innate" preference for a habitat that more closely resembles the more heterogeneous stone substrate. Another possibility is that the slate-bottomed tanks in which these fish were raised more closely resembled the stone substrate as perceived by the fish. A third explanation relates to the more heterogeneous nature of the stone as compared to the sand substrate. Animals generally explore more vigorously, and for a greater length of time, objects that offer more varied or irregular stimulation (Berlyne, 1966).

Comparison of Days One, Two, and Two F

The results for day one, day two, and day two F were markedly different (Tables 1, 3, and 5). There was no indication of positive conditioning on day one (except group five). There are two possible reasons for the random distribution of fish on day one. The fish may still have been in an exploratory phase on day one, but by day two exploration of the available environment was complete and a selection was finally made. This hypothesis is weakened by the lack of a significant difference in activity levels on day one and day two (Table 9). If an exploratory phase prevailed on day one (22 to 24 hours acclimation) and not on day two, there should have been greater activity on day one compared to day two when a selection of a habitat was made.

An alternate hypothesis is that the fish were "stressed" on day one and consequently failed to discriminate or failed to select a habitat. Recent studies have shown the simple capture and handling of fish is sufficient to cause an increase in blood glucose (Chavin and Young, 1970) and changes in plasma protein concentrations, hemoglobin concentration, and erythrocyte size (Bouck and Ball, 1966). Chavin and Young (1970) have shown that the simple net transfer of goldfish from one tank to another produces a significant hyperglycemia of two days duration. Such physiological changes, associated with stress, could be reflected in the behavior of fish. Fish may also be stressed by factors other than handling. Stress may result from being in an unfamiliar habitat. Consequently, fish remaining in an unconditioned habitat would have remained stressed for a longer period of time than fish in a conditioned habitat.

The fact that there was a greater positive effect of conditioning for fish that fed on day two (day two F) than for all fish on day two tends to support the stress hypothesis. Those that fed were presumably the least stressed, and these fish exhibited the strongest habitat preference.

Activity and Color Pattern

Sale's (1969a) hypothesized mechanism of habitat selection predicts that exploration activity should be greater in an inadequate (non-preferred) habitat while non-exploratory patterns should be greater in an adequate (preferred) habitat. His study on juvenile manini, a Hawaiian reef fish, fits these predictions fairly well. To test his hypothesis with the data from this study the activity scores for fish while in the familiar (selected) and unfamiliar habitats were compared (Table 10).

I consider swim time, swim bouts, and pivots as possible measures of exploration. Substrate manipulation seems to be related primarily to feeding and therefore non-exploratory. Based on total activity scores the swim time and substrate manipulation results fit Sale's model, but swim bouts and pivots do not. However, on a group basis, there was no difference in activity frequencies for fish in familiar and unfamiliar habitats. My results do not affirm Sale's hypothesis, but this may be due to several factors. I did not make observations during the first few hours of acclimation, as he did; the species were different; the behavioral measurements were not identical; and in my experiment the fish had a choice of habitats, whereas in Sale's experiment fish were allowed access to only one habitat at a time. Sale made his observations only 65 minutes after a fish was placed in a new habitat. It is interesting that he got significant results with such a short acclimation period.

Regardless of the type of prior experience, the behavior measures of swim time, swim bouts, and substrate manipulations, based on total activity (Table 11), were considerably greater for fish in a sand, than stone, habitat. However, only for substrate manipulations was there a distinct difference based on number of groups. The greater amount of swimming in a sand habitat may be due to the less complex visual appearance of this substrate as compared to the stone. Consequently, more movement might be necessary for sufficient visual stimulation in a sand habitat. The greater amount of substrate manipulation of the sand was probably a result of the nature of the two substrates, since the sand could be picked up, mouthed, and spit out, while the stone could only be scraped.

Stone-conditioned fish swam more, had more swim bouts, and did more substrate manipulating than did sand-conditioned fish (Table 12). This difference between sand and stone-conditioned fish was true regardless of the substrate the stone-conditioned fish were visiting. These results suggest that prior habitat experience can effect subsequent behavior of fish. Other studies have shown that habitat experiences influence behavior of animals. Wecker's (1963) data show that groups of Peromyscus maniculatus given different prior habitat experience had different periods of activity, rate of travel, and depth of penetration into neighboring habitats. Klopfer (1967) and Sheppard, Klopfer, and Oelke (1968) found differences of feeding behavior for island and mainland populations of several bird species.

The greater activity by stone-conditioned fish may be a result of rearing in a more complex habitat, since the stone substrate is more heterogeneous than sand. Luchins and Forgus (1955) reported that rats reared during infancy in varied environments exhibited greater activity in Y mazes when tested at maturity than did restricted rats.

Four-month fish were substantially more active than eight-month fish (Table 13) on the basis of swim time, swim bouts, and substrate manipulations. These results seem reasonable. Greater exploratory activity of young (not infant) compared to older animals of a species has been observed frequently (Welker, 1961).

Pivoting was done at the same rate by four and eight-month fish. As a matter of fact, for all behavioral comparisons (Tables 9, 10, 11, 12, and 13) the pivoting rate was identical or similar. This suggests that pivoting is an important behavior regardless of age, experience, or habitat. During the observation sessions, most of the fish spent the majority of the time resting on or near the substrate in the center of a compartment (habitat), with the "typical" fish occasionally pivoting or, less often, swimming for a few seconds. Perhaps pivoting functions as a monitoring behavior for fish to maintain their "bearings" and perhaps to keep watch for predators, food, etc. Additional work is needed to test this hypothesis.

Neil (1964) found that a hatched color pattern was typical of extreme "fright" in _T. mossambica_. The color pattern analysis (Table 14) indicates that a hatched or striped (often difficult to distinguish from hatched) pattern occurred in a greater (but not significant) percentage of fish in the unconditioned habitat compared to the conditioned habitat. The difference between the conditioned and unconditioned habitat approached significance on day two F. In other words, fish without a hatched or striped pattern were observed more frequently in the conditioned, than the unconditioned, habitat. If the absence of fright colors patterns is an indication of lack of stress, either unstressed fish were more likely to have selected the conditioned habitat, or fish that selected correctly changed to an unstressed color pattern. The latter suggestion is weakened by the fact that I did not observe a change in color pattern as fish changed habitats during the observation sessions. The former suggestion is favored by the findings that: (1) fish selected the conditioned habitat on day two but not on day one, and (2) fish that fed on day two showed a stronger selection for the conditioned habitat than those that did not feed.

T. mossambica as a Representative Fish to Illustrate Habitat Selection

Was *T. mossambica* a good choice to demonstrate fixation of a habitat based on prior experience? There seems to be a basis of argument for both sides. The young of animals that require parental care have a more plastic behavior (Klopfer and Hailman, 1965) and presumably are influenced by experience to a greater extent. Also, fish that remain for a period of time with their parents or parent are more likely to develop responses to environmental stimuli than species with no parental care. Based on this reasoning *T. mossambica* was a good choice.

Klopfer (1965) states that species preferences are proportionally narrower the greater the number of similar species coinhabiting an area.

T. mossambica apparently is found with few if any similar sympatric species and should have relatively wide preferences. The fact that T. mossambica has such a wide distribution and environmental tolerance suggests that this is true. This point suggests that T. mossambica can not be as strongly fixed to one type of habitat as a species with narrower preferences.

The use of a laboratory population for this study may have presented problems. Price (1970) reported that wild populations of P. maniculatus were more hesitant to enter unfamiliar areas than were laboratory populations. Therefore, the use of a field population might have yielded stronger positive results. However, Wecker (1963) found that laboratory stocks of P. maniculatus were influenced much more by prior experience with a habitat than were field stocks. This finding, if it applies to fish, suggests that a field stock of T. mossambica might have been less influenced by early habitat experience.

Applied Aspects of This Research

T. mossambica is an important culture fish in many parts of the world (see Natural History Section). Research on habitat requirements and mechanisms of habitat selection could be useful in the management of this important species.

An understanding of fish behavior, and possible applications of such knowledge, have largely been neglected in fisheries management programs. One example is the put-and-take fishery (primarily trout). Fish are reared in completely unnatural environments with no thought of behavioral needs. They are then placed in streams where they do not act like wild fish. Hatchery-reared trout are very susceptable to hook-and-line fishing, mortality is great, and over-wintering is poor. Perhaps, providing a meaningful environmental experience during a critical period of their development would enable them to respond more like native fish. If early habitat experience is as important to trout as it apparently is to T. mossambica, perhaps only a short exposure to an adequate habitat during the first few weeks would be sufficient to establish desirable behavior of hatchery fish once they are released.

Two experiments (to my knowledge) have been conducted in an attempt to "improve" hatchery-reared trout to increase time at large, increase spread of catch, and reduce mortality. Shetter and Cooper (1957) reported and evaluated an experiment designed to train trout (brook, rainbow, and brown trout) to (I) feed off the bottom, (II) feed off the bottom and avoid predators, (III) feed off the bottom, avoid predators, and use natural cover. The level III trained fish had better spread in the anglers' creel over a period of days than did controls, but the total return of fish was 15 to 20% less than the controls. Unfortunately, most training was done on adult and not young fish. Training of fingerling brook trout (level III) did not increase returns to anglers. But a single planting of fingerling rainbows gave significantly greater returns for level III trained fish over controls. Also, a single planting of brown trout gave improved but not significant returns over controls. It is unfortunate that the experiments with fingerlings were not continued.

Thompson (1966) conditioned juvenile coho and chinook salmon to avoid predators. Trained salmon had significantly reduced mortality. Stomach analysis of predators collected from the study area showed two and one-half as many untrained fish as trained fish in the stomach contents. To my knowledge this study has not been published nor has the procedure been adopted in hatchery operations.

Due to the alteration of aquatic habitats by man, adequate habitat for desirable fish is being reduced. At the same time the demand on these aquatic habitats by sport fishing is increasing. The establishment of sport species in marginal or submarginal habitats would be advantageous. Perhaps by proper manipulation of heredity and/or early experience, populations of desirable species could be established in these habitats.

Hindsight and the Future

The path behind us is often clearer than when it was ahead. In other words, if I could start over again (with additional funds) I would make some changes in the procedures. A more detailed look at each fish in the experimental tanks would have been advantageous. I would suggest the use of photocells and recording equipment to monitor the movement between habitats. This would allow continuous recording, which would reduce the variance between individuals (no 0% or 100% scores); allow analysis of changes of habitat utilization during the course of a day (e.g. before and after feeding); and reduce observer eye strain. Behavioral measures would still have to be done by observation, but the time devoted to each individual could be reduced. Also, I would move the behavioral observations into the first few hours of acclimation. This would allow a better test of Sale's hypothesis. I believe these few changes would have made the results "cleaner".

The list of future experiments suggested by this research is almost endless. A few I consider most important follow. One of the first endeavors should be to determine if the results obtained here can be repeated, first with *T. mossambica* and then with other species. A field investigation of habitat entrainment would be interesting, challenging, and crucial. The next step would be evaluation of habitat conditioning procedures for fisheries management programs.

Several other interesting lines of investigation are open. Of what importance is habitat fixation in nest site selection of *T. mossambica*? How does social hierarchy effect habitat selection in *T. mossambica*? Of what importance are cover, conspecifics, competition, predation, etc. in habitat fixation and selection in *T. mossambica*? How can the effect of habitat entrainment be strengthened (e.g. use of other pertinent habitat cues)? How does stress (e.g. crowding or poor water quality) effect habitat fixation and later selection?

SUMMARY

1. Fish were conditioned (reared) to different habitats and later tested for habitat selection, over a two-day period, in an experimental tank offering a choice of the conditioned habitat (sand or stone) and an alternate habitat (sand or stone). Three regimes of experience were used (early, late, and continuous). Early-experience individuals received experience with the conditioning habitat the first 60 days of life, late-experience the last 60 days before testing, and continuous-experience fish from the time of free-swimming to testing. Within each experience regime, 20 fish conditioned to a habitat with a sand substrate and 20 to a habitat with a stone substrate were tested. Half were tested at four months of age and half at eight months.

2. Early and continuous-experience fish (both four and eight months) significantly selected the conditioned habitat on the second day (but not the first) in terms of total time and number of habitat visits. There was no significant difference between early and continuous experience. There was an age difference for late-experience fish on day two. Four-month fish showed no significant effect of prior experience, but eight-month fish strongly avoided the conditioned habitat on day two.

3. Early experience (before sexual maturity) was found to be not only a sufficient but also a necessary condition for habitat fixation in <u>Tilapia</u> <u>mossambica</u> as judged by a short term selection of two days.

4. The relationship of stress and habitat selection is discussed in light of the findings that: (1) selection was made on day two but not on day one, (2) fish that fed on day two had a relatively strong

habitat selection, and (3) color patterns indicating fright were relatively less common for fish visiting the conditioned habitat.

5. Several behavioral comparisons were made using swim time, number of swim bouts, number of pivots, and number of substrate manipulations. There were no definite differences in activity scores for the comparisons of (1) day one vs. day two and (2) fish in conditioned habitat vs. fish in unconditioned habitat. Fish visiting the sand habitat did more swimming (time and bouts) and substrate manipulating than fish in stone habitat. Stone-conditioned fish did more swimming (time and bouts) and more substrate manipulating than sand-conditioned fish. Likewise, four-month fish did more swimming (time and bouts) and more substrate manipulating than eight-month fish. Pivoting scores were identical or similar for all comparisons, which suggests that this behavior is unaffected by experience, age, or habitat. Pivoting may be necessary for a fish to maintain its "bearings" or awareness. The results of the behavioral comparisons are discussed in relation to other studies.

6. The results are discussed in relation to the natural history of <u>T. mossambica</u> and the possible application of this study to fisheries management.

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