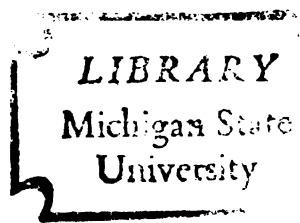EFFECTS OF DEVELOPMENTAL
STAGE AND PRIOR EXPERIENCE ON
HABITAT SELECTION IN THREE SPECIES
OF ANURAN LARVAE

Thesis for the Degree of M. S.
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ABSTRACT

EFFECTS OF DEVELOPMENTAL STAGE AND PRIOR
EXPERIENCE ON HABITAT SELECTION IN
THREE SPECIES OF ANURAN LARVAE

By

Richard Knight O'Hara

Three species of anuran larvae were reared in arti-
ficial habitats that varied only in visual appearance. At
different points in development, samples of single indi-
viduals from each habitat were allowed to choose between
two conditioning substrates to determine the influence of
developmental stage and prior experience on habitat selec-
tion. The design of the two standard test substrates
allowed discernment of the relative importance of midwater
and bottom cues in selection of a substrate. Results showed
highly stereotyped behavior dependent primarily on cues
situated in the vertical plane (midwater).

In a preliminary study, larvae of the wood frog,
Rana sylvatica, were reared from the hatchling stage in two
experimental habitats. Habitat A was composed of alter-
nating vertical black and white stripes affixed to the
sides and a uniform white bottom. Habitat B consisted of
identical black and white striping on the bottom and uniform

white sides. Following 20-25 days experience, when presented with a choice between Habitats A and B, larvae conditioned in Habitat A (Group A) significantly selected Habitat B in both initial choice and total time. Habitat B conditioned larvae (Group B) also demonstrated preference for Habitat B in distribution of total time. During testing, spontaneous reactions by individuals of both groups when Habitat A was approached indicated that this selection was based on an avoidance to the vertical stripes of that habitat.

Larvae of the American toad, Bufo americanus, were reared from the day of hatching through metamorphosis in experimental Habitats A and B and a uniform white control habitat (Habitat C). No experiental or developmental influence was indicated on days 9-11 and 23-25 posthatching when larvae of all three groups demonstrated preference for Habitat B in initial choice and total time. Swimming behavior indicated strong avoidance to the vertical stripes of Habitat A and activity for every group was much greater while over the preferred substrate. This relation of activity and habitat is discussed in reference to other studies.

Tadpoles of Groups B and C were again tested for preference on days 34-37 when they exhibited varying stages of transformation. Larvae of both groups continued to avoid Habitat A in initial choice and total time. However, those with tails resorbed to 7 mm or less reversed choice

behavior and strongly selected Habitat A. This sudden developmental change seems to represent strong attending to cues resembling a climbable surface and is substantiated by swimming behavior within Habitat A during testing.

When B. americanus larvae were presented with a choice between Habitat B and Habitat C on days 32-33, groups tended to prefer Habitat C. This substantiated indications that the side striping of Habitat A was the avoided component and suggested that the bottom striping was not only discriminated, but was also aversive in a lesser degree.

Larvae of the green frog, Rana clamitans, were reared from hatching in the three standard conditioning habitats and tested for preference throughout development. On days 10-11, all groups significantly chose Habitat B in initial choice. Group A tadpoles continued to demonstrate preference for Habitat B on days 25-28, however, Groups B and C exhibited no preference. It appears that a general developmental change in preference occurred at this stage and prior exposure of Group A to Habitat A was sufficient to overcome this change. On days 52-54, Groups A and B tended toward selection of Habitat B, but not significantly. Group C strongly preferred Habitat B in this test and again on day 66. The final standard test on days 81-83 post-hatching showed Groups B and C avoiding Habitat A while Group A indicated an influence on selection attributable to prior conditioning by exhibiting no preference.

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A variety of novel substrates, substituted in place of Habitat A, were tested with R. clamitans in an effort to determine the "aversive" components of the vertical stripes. Aversive visual flicker was eliminated as a factor by testing with horizontal stripes. Overall reflective intensity of the substrate was likewise discarded as a single influencer of selection by testing uniform gray and uniform black sides. Tadpoles did not actively discriminate vertical segments of aquatic vegetation (Elodea canadensis) and when horizontal "fringe" was added to the black stripes of Habitat A, tadpoles continued to strongly avoid that substrate. By manipulation of widths and ratios of the vertical black and white stripes, it was concluded that the ratio of the contrasting stripes was important in eliciting avoidance to Habitat A in this species. Adaptive implications of these behaviors are discussed.

EFFECTS OF DEVELOPMENTAL STAGE AND PRIOR

EXPERIENCE ON HABITAT SELECTION IN

THREE SPECIES OF ANURAN LARVAE

By

Richard Knight O'Hara

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INTRODUCTION

Within the geographic range of a species, animals are neither uniformly nor randomly distributed. Rather, their spatial patterning is associated with particular features of the immediate environment, the bounds of which are largely dependent upon interactions with other individuals and species and certain environmental constraints. Factors such as competition and predation as well as a species' physical limitations and physiological tolerances are recognized as primary influences acting to restrict animals to defineable habitats. Until relatively recently (Lack, 1933), little attention has been directed at possible psychological influences under which single or varied environmental parameters are responded to preferentially. Habitat selection as such implies discrimination and active preference and should not be confused with habitat correlation (Klopfer, 1969).

Considerable evidence offered in support of habitat selection in vertebrates is of a correlative and indirect nature. There is difficulty in directly dealing with a problem of such complexity which necessarily demands

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simplistic procedural analysis in demonstrating its actuality and defining its primary components. Experimental procedure in standard design allows an animal to actively choose between two or more "habitats" differing in one or more characteristics. The selected habitat is designated as the one initially selected or the one in which the majority of time is spent.

Experimental studies of this type conducted in highly simplified artificial conditions yield questionable results in terms of relevance to the natural situation. However, such an approach can enable an experimenter to begin to assess an animal's discriminative capabilities, the class and components of conditions preferred or avoided, and the underlying basis for the selective process itself. Coupled with field observations, predictions can be made about an animal's behavior in certain natural circumstances and ideas formulated concerning its adaptiveness.

Much of the recent work investigating habitat selection is with fish and indicates a variety of potential parameters affecting preference. Sale (1968 and 1969a) reports several proximate factors influencing habitat selection in the juvenile manini, Acanthurus triostegus. The presence of substratum, cover, food algae, and the depth of the water were all of importance. Plant morphology was apparently a strong determinant of distribution within a stream for fourspine sticklebacks (Baker, 1971). Hagen (1967) investigated mechanisms acting to isolate two

subspecies of the threespine stickleback, <u>Gasterosteus</u>
<u>aculeatus</u>, in a stream. Results of field observations and
preference tests showed distinct differences in preference
of type of nesting substrate, nesting vegetation type, and
selection of water current. Other investigations indicate
that factors such as overhead cover, light intensity,
shelter complexity, water velocity, depth, and turbulence
may be important cues to some fish in selecting micro-
habitats (Hunsaker & Crawford, 1964; Kwain & McCrimmon,
1967; McCrimmon & Kwain, 1966; Butler & Hawthorne, 1968;
Haines & Butler, 1969; Baldes & Vincent, 1969; and Sale,
1972).

Hypothetical mechanisms of habitat selection and
their ontogeny have been discussed in reference to fish
(Sale, 1969b) and birds (Svardson, 1949; Hilden, 1965; and
Klopfer & Hailman, 1965) but offer little substantive
experimental data. Preliminary work has sought to demon-
strate the reality of a selective process and define the
variables capable of modifying and structuring it. Testing
of the underlying basis of habitat selection has been
incorporated into several recent studies. It appears that
learning may play a significant role.

Sale (1971) presented representatives of two popu-
lations of the reef fish, <u>Dascyllus</u> <u>aruanus</u>, with a choice
between two species of coral that each group was found to
frequent and a third "less desirable" species. Fish
selected to occupy the coral from which they were collected,

indicating that prior experience may have affected subsequent preference. Early experience within the first 60 days of life was a necessary prerequisite for subsequent selection of a stone or sand substrate in the African cichlid, _Tilapia mossambica_ (Quertermus, 1972), but does not influence nest-site selection which is based on the substrate of smallest particle size (Thoms, 1974).

Klopfer (1963, 1965) and Klopfer and Hailman (1965) studied experiential influences on foliage preference in small numbers of chipping sparrows. Although choices seemed primarily genetically dependent, there were indications that learning through early experience may have modified later foliage preference. Sargent (1965) showed that the conditions under which young zebra finches were reared affected selection of nestling materials and habitat in mature individuals.

Studies with mammals have indicated a strong hereditary basis for selection of a habitat. In field and woodland subspecies of the deermouse, _Peromyscus maniculatus_, Harris (1952) found that mice preferred habitats resembling their natural environment even without prior outdoor experience. Harris concluded that this behavior was innate, although possible experiential influence was untested. Wecker (1963) studied this problem in greater depth with the prairie deermouse, _P. m. bairdi_. Habitat choice of its natural field habitat was primarily hereditary as early experience in a woods or laboratory habitat did not modify

preference for the field habitat. Animals reared for 12-20 generations in the laboratory lost their genetically based preference, but retained the ability to reestablish preference following experience in a field situation.

Psychological preference for a habitat seems a profound influencer of microdistributional patterns in many vertebrates. There is, however, little basis for such an assertion in regard to the amphibians. Supportive evidence of habitat selection is mostly correlative and lacking in experimental data.

In review of the literature, it seems that any selection process occurring in adult amphibians is most probably dependent on critical moisture and temperature levels or in locating suitable breeding and nesting sites. Heatwole (1962) reports that the microdistribution of the red-backed salamander, Plethodon cinereus, is dependent on temperature, humidity, and log and litter type. The narrow-mouthed toad, Microhyla carolinensis, is limited by moisture and cover, with distribution and density correlating with plant associations and the depth of penetrable organic layers (Anderson, 1954). Ralin (1968) found indications that microclimatic differences in relative humidity was sufficient to separate two sympatric species of hylids ecologically and reproductively. Moisture also seems to govern the distribution of the cave salamander within the twilight zones of limestone caves (Hutchison, 1958).

Heatwole (1961) investigated local distribution patterns of the wood frog, Rana sylvatica, in southern Michigan. Locations were restricted almost entirely to moist, forested areas. Arborescent vegetation providing shade in conjunction with a nearby body of water seemed important. When tested experimentally, selection of litter structure was influenced by substrate moisture.

The influence of temperature on substrate selection in different developmental stages of the clouded salamander, Aneides ferreus, was studied by McKenzie and Storm (1970). Young salamanders selected bark litter over all temperatures, subadults selected bark litter only at higher temperatures, and adults exhibited no preference between bark litter or a rocky substrate.

The specificity with which breeding and oviposition sites are selected, particularly in the anurans, has been commonly observed. Breeding site locations and oviposition sites are often correlated with such factors as the size, permanence, and quality of a body of water, the character- istics of the surrounding terrain, aquatic vegetation, and water depth. Bragg (1940a) and Bragg and Smith (1942) when studying the ecology of Bufo cognatus, noted breeding to be confined to clear, shallow water. While calling males were indiscriminate in choice of a mating location, females were very selective in choosing larger, longer-lived pools. Breeding, nest site selection, and local segragationing of overlapping breeding species are reviewed for many anurans

of Oklahoma by Bragg (1940b) and Bragg and Smith (1942).
Interesting to note is the observation by these authors
that the toad <u>Bufo</u> <u>woodhousei</u> usually calls while sitting
in the water, whereas <u>B</u>. <u>americanus</u> when breeding at the
same time will call from the banks. The opposite condition
was observed by Brown and Pierce (1965) in southern Illi-
nois.

A few studies report greater specificity of nest
site selection than correlation with the gross features of
water-type and location. Field observations suggest that
the eggs of the northern chorus frog, <u>Pseudacris</u> <u>triseriata</u>,
are laid on slanting vegetation (Wells, 1924) while <u>P</u>.
<u>clarkii</u> lays its eggs on upright vegetation (Bragg, 1943).
Sexton and Ortleb (1966) cite experimental evidence
indicating that the leptodactylid frog, <u>Engystomops</u>
<u>postulosus</u>, responds to shallow water and a curved surface
or a solid vertical plane intersecting and perpendicular to
the surface of the water.

Perhaps the least understood period in the life of
amphibians is the larval stage. This is, however, where
the greatest mortalities are sustained and primarily where
the size of the subsequent breeding population is ultimately
determined. Predation on larvae is heavy, and severe inter-
and intraspecific competition for critical factors such as
food and oxygen and the process of growth inhibition can
become critical at high densities, retarding development
and indirectly or directly causing mortality (Savage, 1952;

Brockelman, 1969; Wilbur, 1972; and Wilbur & Collins, 1973).
A possible method for lessening the detrimental effects of
such interactions might be development of a microhabitat
preference. This would certainly be advantageous in cir-
cumstances where two or more species are developing in the
same body of water and could function to decrease inter-
actions with predators.

Microdistributional data and information about
ecological relationships of anuran larvae are severely
lacking. Bragg (1940a) observed that tadpoles of Bufo
cognatus were restricted entirely to shallow waters at one
end of a pond where breeding in the adults was previously
limited. Larvae of B. canorus appeared to congregate in
warm shallow waters of a pond during the day and deeper
waters at night (Mullally, 1953). Brockelman (1969) reports
the distribution of B. americanus tadpoles to be restricted
within 2.5 meters of the water's edge in lakes of southern
Michigan but to be unrestricted in marsh habitats. Most of
the somewhat rare ecological observations of anuran larvae
are of this incidental type, with little concern as to the
variables that may take part in localizing different
species.

A more thorough study was accomplished by Heyer
(1973) on several sympatric species of anuran larvae in
Thailand. Relative environmental segregation was main-
tained not only through utilization of different ponds, but
also by general spatial partitioning of the living space

within a pond. However, possible variables governing this distribution were not discussed.

Temperature can be a very important factor to amphibian larvae in lessening time to transformation, often having substantial influence on subsequent population size. Quite specific temperature selection by anuran larvae in the laboratory and the field has been demonstrated repeatedly (Workman & Fischer, 1941; Brattstrom, 1962; Herreid & Kinney, 1967; Lucas & Reynolds, 1967; and Vlaming & Bury, 1970).

Substrate type selection by the tailed frog, Ascaphus truei, was investigated experimentally by Altig and Brodie (1972). When tested in an artificial stream, tadpoles preferred substrates of smooth stones of specific diameter. Selection of such a substrate in fast-flowing streams is probably not entirely of psychological choice but seems related to the structure and size of the oral disc. Tadpoles were also positively rheotactic and negatively phototactic.

Few studies have attempted to examine learning ability in larval amphibians, and this potential is apparently regarded with pessimism. Munn (1940) demonstrated that small numbers of Hyla cinerea, H. versicolor, and Rana catesbeiana were capable of learning Y and T mazes. Also, tadpoles of Hyla arborea performed simplistic tasks of form discrimination (Pache, 1932).

Until recently, no substantive experimental data showing ability of anuran larvae to visually perceive and prefer particular habitats nor its underlying basis was available. Preliminary study into these questions was initiated by Wiens (1970 and 1972) using two species of Rana conditioned in uniform striped, square-patterned, and featureless substrates. Tadpoles of the red-legged frog, R. aurora, reared in featureless or square-patterned habitats showed no preference at any time for either striped or square-patterned substrate while stripe-reared larvae significantly preferred the striped habitat (Wiens, 1970). This preference, established within the first two weeks of conditioning was retained after isolation from the conditioning substrate and could readily be renewed in young and old tadpoles. In a similar experiment (Wiens, 1972), R. cascadae tadpoles demonstrated selection only following conditioning in the square-patterned substrate. Observations of the developmental ecology of these species indicate that these learned preferences may reflect the natural larval habitat.

The present study was undertaken on several bases. There exists considerable evidence supporting habitat discrimination and selection of breeding and oviposition sites in adult anurans. Some anuran larvae are capable of discerning and selecting certain environmental characteristics (e.g., temperature) and have been observed to exhibit a limited degree of species specific spatial segregation.

The ability to learn simple visual cues has been demonstrated in some tadpoles and experientially-dependent substrate preference was shown to occur in two species of frog larvae. Indeed, habitat selection of a learned or genetic basis could serve as an important mechanism in lessening pressures arising from predation or competitive interaction or assuring rapid, healthy development.

It was the design of this investigation to rear anuran larvae in three artificial habitats differing only in visual characteristics and to test subsequent preference of two of these habitats at the earliest possible date and throughout development. In addition to observation of developmental and experiential influences, it was hoped some insight into the visual components of the habitats responsible for any stereotyped choice pattern could be deduced in addition to the relative importance of midwater and bottom cues.

Three local species of anurans were chosen for this study. Tadpoles of the wood frog, Rana sylvatica, were used in a preliminary experiment to determine the effectiveness of the chosen visual parameters in producing a response. Wood frogs are found in relatively mesic forests in close proximity to water. Breeding occurs in late March through April in transient woodland pools were eggs are attached to vegetation or submerged objects in spherical masses. Transformation is completed in six to 12 weeks.

A second series of experiments employed larvae of the American toad, <u>Bufo</u> <u>americanus</u>, and investigated experiential and developmental influences on choice behavior using visual parameters identical to those of the previous test. Adults of this species are found in more xeric habitats in grasslands, sparse woods, and frequently in gardens and farmland areas. Breeding is from late March or April and may extend as late as July, with peak breeding in mid-April. Larvae normally metamorphose in six to 10 weeks.

Tadpoles of the green frog, <u>Rana</u> <u>clamitans</u>, were used in a final series of tests. These were subjected to the same rearing experiences and in addition to testing for experiential and developmental affects, the pertinent visual component of the experimental substrate was investigated. <u>R</u>. <u>clamitans</u> inhabit swamps, larger ponds, and sometimes streams, always in close association with water. Eggs are laid from May into August in large flattened masses in the surface film amid emergent and floating vegetation. Eggs laid early in the season may transform by early fall while others overwinter and metamorphose the following year.

METHODS AND MATERIALS

Species Selection

Choice of anuran species for experimentation entailed three basic determinants: (1) the ease with which eggs could be obtained at the time of an experimental series; (2) the degree to which each species was related to the other; and (3) the type habitat in which each species, adults and larvae, is frequently found.

Rana sylvatica is among the first frogs to emerge from hibernation and to commence breeding. These not only provided an excellent opportunity for early testing of experimental design and parameters, but offered an interesting alternative in habitat type. Larval development most frequently takes place in shallow, transient, woodland pools quite devoid of any substantial vegetative growth.

Bufo americanus, the most xeric of the forms tested, begins breeding quite early and may continue through June or July. Choice of oviposition sites is somewhat indiscriminate, thus exposing larvae to varied aquatic habitats. Development usually extends early into the summer season when pond characteristics (e.g., vegetation) have changed from those present in the spring.

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Rana clamitans breed throughout most of the summer months in the more permanent, swampy areas. Larvae may overwinter and are consequently subjected to nearly all stages of seasonal pond succession.

Choice of Experimental Visual Parameters

In selecting a visual parameter, the primary objective was not to reproduce a natural component of the larval aquatic habitat. But rather to create a readily distinguishable habitat, simplistic in design, that would test the comparative relevance of midwater versus bottom cues. However, in view of Wien's (1970) results, suggesting that a striped pattern may have resembled linear vegetation to Rana aurora tadpoles, stripes were chosen as the experimental pattern for the present study. Indeed, one of the only obvious visual characteristics that differs within and between southern Michigan ponds is vegetation type and density.

Experimental Habitat A was composed of a white, featureless bottom and alternate one-half inch wide black and one-half inch wide white vertical stripes fastened to all sides of the tank. Habitat B consisted of identical striping on the bottom of the tank and white sides. Control Habitat C was composed of uniform white sides and bottom.

Conditioning and Testing

Rana sylvatica

On 31 March 1973, several hundred eggs from multiple clutches of the wood frog, R. sylvatica, were collected in a woodland pool in Meridian Township, Michigan, approximately one-fourth mile north of M-43 and one-fourth mile west of Meridian Road. These animals were to be used in a preliminary study to test methods and to determine if the chosen visual parameters could be discriminated and differentially treated.

Hatching commenced and was completed on 4 April, and approximately 50 larvae were transferred to each of two glass aquaria filled to a depth of 20 cm (26 liters) with dechlorinated tap water. Aeration was provided by air stones. To the base and sides of each tank was affixed adhesive, white plastic vinyl to the height of the water-line. In rearing tank A, one-half inch wide black vinyl strips were attached vertically to all sides at one-half inch intervals. Stripes of the same width and interval were positioned widthwise on the base of rearing tank B. Larvae were fed rabbit pellets or "TetraMin Staple Tablet Food" twice daily and water was changed every other day. Photoperiod was uncontrolled and temperature fluctuated between 20 and 23°C. On days 20-25 posthatching, larvae were tested for preference.

Bufo americanus

On the night of 10 May, 1973, one clutch of approximately 5,000 eggs was collected from the west end of a permanent pond located in a field immediately south of the Natural Resources Building at Michigan State University, East Lansing. Until hatching, eggs were placed in one standard control rearing tank. All conditioning tanks were structured of three-fourth inch marine plywood, sealed with gray epoxy paint, and measured 87.5 x 57.0 x 30.5 cm. Each was uniformly lined with white plastic sheeting. Tanks were filled with charcoal filtered water (100 liters) to the height of the plastic sheeting (20 cm) and aerated with single air stones. Black vinyl strips were affixed to Habitats A and B as previously described.

Hatching was completed on 13 May, when larvae were transferred to respective rearing habitats. These consisted of one tank each of types A and B and three featureless, white control tanks (C). Ninety larvae were placed in each habitat. All but one control tank were positioned on a stand directly beneath a uniform double row of 40W cool white lights. Lights were suspended 60 cm above the water surface. The remaining control tank was situated on a stand 61 cm below the other tanks. This third control tank was employed as a possible reserve resource of animals and for possible use in subjecting larvae to subsequent periods of varied experiential treatment or retention tests.

All conditioning tanks were placed on a controlled light regime of 16L:8D (on at 800 hours and off at 2400 hours). Laboratory temperatures normally fluctuated between 21 and 23°C but reached as high as 26°C during the last 10 days of the experiment. Tadpoles were fed "TetraMin Staple Tablet Food" twice daily and all fecal material, uneaten food, and most water was siphoned out of every rearing tank at two day intervals.

A total of four tests were initiated on days 9, 23, 32, and 34 posthatching with each test period spanning 2-3 days. In all tests except Test III on day 32, individuals were presented with a choice between habitat types A and B (also designated Side A and Side B) to determine developmental and experiential effects. Test III substituted Side A with an all white control habitat. Experimentation was terminated at metamorphosis.

Rana clamitans

On 4 July, one clutch of recently deposited eggs of the green frog, R. clamitans, was collected in the northwest corner of a marshpond in the Rose Lake Wildlife Research Area, 12 miles northeast of Lansing, Michigan. This pond was located in the center of the Orange Area (between Woodbury and Peacock Roads) just southwest of the region of flooding. One-hundred twenty eggs were immediately placed in each of the five rearing tanks previously described. Hatching occurred on 6 July, and larvae were henceforth

reared under the same procedures as employed with larvae of
B. americanus.

To determine if conditioning and/or developmental
stage had any influence on habitat choice, all three groups
were tested for preference of Habitats A and B on days 10,
25, 52, and 81 posthatching. A standard test was also
carried out on day 66 with Habitat C reared larvae for
indications of possible change in behavior during that time
interval.

In view of results obtained with R. sylvatica and
B. americanus, it was decided to increase numerically the
larvae in each conditioning habitat to enable investigation
of the primary component of the visual parameter employed
that was effecting observed responses. During time inter-
vals between standard tests, several tests were performed
varying the visual characteristics of the habitats. These
comprised altering widths and proportions of stripes of
Habitat A, changing pattern, and testing the affects of live
aquatic vegetation (Elodea canadensis) on habitat selection.
In the later test, vertical segments of Elodea extending to
the water surface were arranged in three staggered rows in
two-thirds of one side of the test tank. Rows were 5 cm
apart and individual segments were placed at 10 cm inter-
vals within each row. A second test approximately doubled
this density. Experimentation was terminated on day 96
posthatching when numbers were depleted.

Test Apparatus and Procedure

The test tank (Figure 1) measuring 57 x 57 x 30.5 cm was divided equally into the two rearing substrates, A and B, and filled with 65 liters of charcoal filtered water to the depth of the substrate (20 cm). One light unit containing two 40W cool white lights was centered 78 cm directly above the water surface of the tank. Lights and test arena were completely enclosed with a dark, opaque curtain with a single, narrow slit through which observations were made.

Standard testing procedure was established following experimentation with methods during preliminary testing of R. sylvatica. In testing of B. americanus and R. clamitans larvae were randomly chosen for testing in sequential series from respective habitats to avoid clumping of individuals of one rearing habitat at any particular time period or day of testing. Tadpoles were placed singly into a 10 cm diameter plexiglas cylinder, open at both ends, and situated vertically in the center of the test tank. After a given time period (10 minutes) and when the tadpole was motionless on the bottom, orientation within the release chamber was recorded. The release chamber was then lifted to the top of the enclosure by external means and behavior of the tadpole was observed for a 10 minute period. The side to which initial movement was directed and into which the tadpole penetrated approximately 6-8 cm or beyond was defined as an initial habitat choice. Total time and

Figure 1. Experimental test tank showing Habitats A and B.

activity spent in each habitat were measured using two
"GraLab" timers and one stop watch. The number and direc-
tion of side changes and comments regarding general behavior
were noted. Following testing the total length of each
individual was recorded before discarding from the experi-
ment. At the end of each day of testing, the sides within
the test tank were reversed to control for the influence of
cues internal to the testing apparatus.

Statistical Treatment of Data

Four measurements used to indicate habitat prefer-
ence were subject to statistical analysis. Goodness of fit
for distributions of pre-release orientations and initial
choices were analyzed using a chi-square test. Variation
from 50 percent within groups of percent total time spent
over one substrate were analyzed by use of a t-test (Sokal &
Rohlf, 1969). A single classification analysis of variance
was calculated on total times spent per substrate between
groups within a test. To determine if activity times
varied with experience or test substrate, one factor two-
way analyses of variance with replication (Sokal & Rohlf,
1969) were run on the percent of the total time over each
habitat that was spent in activity.

RESULTS

Testing of Rana sylvatica

Larvae of Rana sylvatica were conditioned in
respective habitats (50/tank) and tested for preference on
days 20-25 posthatching. Substantial mortalities occurred
during rearing in each group. The cause for the mortality
is unknown, however, largest numbers were lost immediately
following water changes suggesting that either temperature
or mechanical disturbance was responsible. In total, 33
larvae conditioned in a habitat of striped sides and
featureless base (Group A) and 18 conditioned in a habitat
of featureless sides and striped base (Group B) were tested
in a test tank bearing equal proportions of each substrate.

Larvae of Group A were tested on days 20-24 post-
hatching. Average total length at this period was 27.2 mm.
Orientation within the release chamber immediately prior to
release did not vary between sides as 12 of the tadpoles
oriented toward Substrate A and 13 toward Substrate B.
However, only 7 of 25 larvae tested initially selected Sub-
strate A. This was significant at the 5 percent level when
subjected to a chi-square test.

Following periods of 10-20 minutes within the release chamber, individuals A_1 through A_{10} were released and observed for 12 minutes and larvae A_{11} through A_{25} were observed for 15 minutes. (There were no observable differences in measured parameters due to varied test period lengths.) Larvae spent on an average 28 percent of their time within Habitat A and 72 percent within Habitat B. Individual values over each substrate varied significantly from random ($p < .001$) when subjected to a t-test.

Time of activity per side was recorded for individuals A_{11} through A_{25}. Expressed as a percentage of respective total times, equal values of 21 percent were calculated as time spent swimming over each substrate. Side changes ranged from 0 to 8 with a mean of 2.

As an additional test of the strength of initial choice as an indicator of preference, four individuals were released from the center of Habitat A and four from the center of Habitat B (A_{26}-A_{33}). Three of the tadpoles released from Habitat A initially selected Habitat B and all four released from Habitat B remained in that side.

A mean of 25.2 mm was recorded for total length of Group B larvae. Of 18 tested, nine were released from the center of the test tank and nine from the center of Habitat A. In neither case did larvae show significant orientation prior to release. Seven released from the center of the test tank initially chose Habitat B. This was not significant; however, percent total times of 8.4 and 91.6 for

Sides A and B respectively were highly significant (p <
.001). Percent active time for this group was 19.7 percent
in Side A and 13.2 percent in Side B. Side changes ranged
from 0 to 4 with a mean of 2.

No significant differences were noted in any cate-
gory for nine larvae released within Habitat A. Six ini-
tially chose Side B and average total times of 44.9 percent
and 55.1 percent were apportioned to Sides A and B. Only
5.2 percent and 9.7 percent comprised active movement and
overall side changes decreased to one.

Behavioral observations recorded for each indi-
vidual tested support the indication that Habitat B was the
preferred substrate of both groups and suggest that larvae
may have actually been avoiding the habitat with striped
sides. It was commonly observed in both groups that tad-
poles approaching Side A from Side B would either quickly
reverse their direction of movement upon reaching the
border, actually enter Side A for a very short distance
before returning to Side B, or enter Side A and stop within
4-5 cm of the border and remain motionless on the bottom
before ultimately returning to Side B.

Although unreflected in orientation prior to
release, a considerable proportion of tadpoles, predomi-
nately of Group B, exhibited persistent swimming movements
toward Side B during the period spent within the release
chamber.

Testing of Bufo americanus

Because of clear discrimination and selective be-
havior demonstrated by R. sylvatica, identical visual cues
were retained for controlled testing of Bufo americanus
larvae.

Through development, three standard tests of prefer-
ence of Substrates A and B were conducted. Summary of these
data is given in Table 1, and statistical treatment of
initial choices and total times is presented in Table 2.
Total time over each test substrate is given as a percent-
age of a ten-minute test period. Activity times are in
percents of total time over that substrate. Pre-release
orientation was randomly distributed for every group in
each test and is not included in data summary.

Test I was initiated at 9 days posthatching at
stages 24-25 (Gosner, 1960) when larvae began to show
coordinated swimming movements, feeding activity, and eyes
appeared functionally developed. Total lengths were not
measured for every individual in this test but random
measurements indicated lengths of 12-14 mm for these groups.
A very strong preference for Side B as evidenced by initial
choices and distribution of total time was clearly demon-
strated by Groups A and C. Only Group B showed no prefer-
ence of first side choice behavior. But this does not seem
a true representation of behavior as over 80 percent of the
total time was spent in Side B.

Table 1.--Mean data distribution of initial choices, total time, activity, and side changes in standard testing of B. americanus tadpoles.

Test	Days (Posthatching)	Group	T.L.(mm)	Init. Choice A	Init. Choice B	Total Time(%) A	Total Time(%) B	Activity(%) A	Activity(%) B	Side Changes
I	9-11	A		1	14	14.5	85.5	46.0	68.0	7
		B		4	11	18.1	81.9	22.8	60.5	6
		C		2	13	21.5	78.5	26.9	62.4	8
II	23-25	A	15.0	2	18	32.7	67.3	32.3	43.5	9
		B	15.2	4	16	27.6	72.4	27.5	55.3	10
		C	15.4	1	19	28.4	71.6	28.2	50.1	11
IV	34-37	B	23.5	2	15	21.9	78.1	8.3	11.6	2
		C	23.5	4	17	29.2	70.8	7.2	11.8	2
		B'	13.1	8	1	84.5	15.5	10.9	6.7	1
		C'	13.8	11	0	93.2	6.8	4.5	1.1	1

Table 2.--Probability values of initial choice distributions (chi-square test) and percent total time spent over respective substrates (t-test) by B. americanus larvae.

Test	Group	Initial Choices	Total Time(%)
I	A	p < .001	p < .001
	B	p < .50	p < .001
	C	p < .01	p < .001
II	A	p < .001	p < .001
	B	p < .02	p < .001
	C	p < .001	p < .001
IV	B	p < .005	p < .001
	C	p < .01	p < .01
	B'	p < .05	p < .001
	C'	p < .001	p < .001

Approximately two weeks subsequent to Test I, a second test was run to determine if further conditioning or development may have influenced choice behavior. No effect was evidenced and all groups continued to strongly select Substrate B in both categories of time and first choice.

As a consequence of a sharp rise in laboratory temperature soon after Test II was completed, tadpoles developed very rapidly necessitating premature testing. At the time of Test IV, all stages of metamorphic transformation were present, but due to losses from mortality and metamorphosis an insufficient number of Group A tadpoles were available for testing. Representative samples across all stages were tested in an attempt to differentiate any late developmental affects on habitat selection. The most measureable characteristics during this period are the degree of hind- and forelimb development and body-tail proportions. During stages 43-44 (Gosner, 1960) when larvae become frog-like and are nearing the juvenile, terrestrial stage, a distinct reversal in habitat preference ensues. Tadpoles with tail lengths measuring greater than 7 mm continued to strongly select Substrate B. However, at tail lengths equal to or less than 7 mm (Groups B' and C'), previous behavior was reversed and Substrate A became the preferred habitat.

It is apparent that rearing experience had no influence on initial choices in any test. To determine if there was variation in distribution of the percent total times

among groups, one-way analyses of variance were calculated
and are presented for Test I, II, and IV in Tables 3, 4,
and 5 showing sum of squares, mean squares, and F-values.
Results in all cases show total times independent of prior
habitat experience.

Activity over Substrates A and B in Tests I and II
was evaluated for dependent variables by subjecting data to
tests of two-way analyses of variance with replication.
Activity was designated as the percent of the total time
over each substrate spent swimming. For Test I, Table 6
indicates effects arising from two sources. However, group
rearing experience, shown to be an influencing factor, is
misleading. A review of the data showed variation pre-
dominately due to four individuals (three in Group A and
one in Group B). In these cases only three to 26 seconds
were spent in Habitat A of which the entire time was spent
active. This gave values of 100 percent activity over that
substrate, thus biasing values for that group. Greatest
significance resulted from test substrate. Larvae of all
groups spent proportionately much less time active over the
avoided substrate (A). No interaction of experience and
test substrate was indicated. Table 7 illustrates similar
findings with activity data of Test II. Only test substrate
was responsible for variation. Because of minimal activity
in later stages, it was not possible to accurately evaluate
activity for larvae of Group IV.

Table 3.--One-way analysis of variance of percent total
time spent in Side A by B. americanus during
Test I.

Source of Variation	df	SS	MS	F	
Among Groups	2	385.86	192.93	0.917	ns
Within Groups	42	8840.39	210.48		
Total	44	9226.25			

Table 4.--One-way analysis of variance of percent total
time spent in Side A by B. americanus during
Test II.

Source of Variation	df	SS	MS	F	
Among Groups	2	296.78	148.39	0.624	ns
Within Groups	57	13,557.28	237.85		
Total	59	13,854.06			

Table 5.--One-way analysis of variance of percent total
        time spent in Side A for B. americanus tadpoles
        with tail lengths greater than 7 mm and less than
        or equal to 7 mm in Test IV.

Source of Variation	df	SS	MS	F	
> 7 mm					
Among Groups	1	490.86	490.86	0.784	ns
Within Groups	36	22,532.57	625.90		
Total	37	23,023.43			
≤ 7 mm					
Among Groups	1	374.04	374.04	1.687	ns
Within Groups	18	3,990.55	221.70		
Total	19	4,364.59			

Table 6.--Two-way anova with replication of the percent
        total time spent active by B. americanus over
        Habitats A and B in Test I.

Source	df	SS	MS	F	
Subgroups	5	25744.72	5148.94		
A (Experience)	2	5445.58	2722.79	4.170	p < .025
B (Habitat)	1	18454.18	18454.18	28.261	p < .001
A X B	2	1842.56	921.28	1.411	ns
Within (error)	84	54852.45	653.00		
Total	89	80594.77			

Table 7.--Two-way anova with replication of the percent
        total time spent active by B. americanus over
        Habitats A and B in Test II.

Source	df	SS	MS	F	
Subgroups	5	14210.23	2842.05		
A (Experience)	2	158.68	79.34	0.171	ns
B (Habitat)	1	11818.69	11818.69	25.421	p < .001
A x B	2	2232.86	1116.43	2.401	ns
Within (error)	114	53000.54	464.92		
Total	119	67210.77			

Results and behavioral observations of Tests I and
II suggested that the vertical stripes of Substrate A were
aversive, thereby affecting selection. In an effort to
resolve if there was actually positive orientation toward
Substrate B, Substrate A was replaced with a uniform white
substrate (C). Results and their significance are presented
in Table 8. Substrate B was clearly not preferred. On the
contrary, initial choices of Groups B and C and total time
distribution of Group B demonstrated preference for Sub-
strate C. Analysis of variance of total times (Table 9)
indicated no differences between groups.

Behavioral Observations

Each individual tested was observed and noted for
any stereotyped behavioral patterns in the test situation.
In Test I, avoidance behavior similar to that observed in

Table 8.--Mean data distribution of initial choices, total time, activity, and side changes by B. <u>americanus</u> between Habitats B and C in Test III.

Days	Group	T.L.(mm)	Init. Choice		% Total Time		% Activity		Side Changes
			C	B	C	B	C	B	
32-33	A	26.1	8	3	56.1	43.9	36.3	30.0	10
	B	25.1	13	1*	65.2	34.8***	18.6	18.7	4
	C	25.4	12	2**	51.1	48.9	31.0	25.1	9

*p < .005.

**p < .025.

***p < .05.

Table 9.--One-way analysis of variance of percent total
time spent in Side C by B. americanus during
Test III.

Source of Variation	df	SS	MS	F	
Among Groups	2	1,405.96	702.98	1.741	ns
Within Groups	36	14,538.54	403.85		
Total	38	15,944.50			

R. sylvatica tadpoles was recorded, but in greater fre-
quency. Most of the time was spent along the extreme side
of Side B. Approaches to Side A and subsequent reversals
at the border were very common. Actual entries into Side A
most frequently resulted from approaches away from the sides
and toward the center of the tank. Less often, were pene-
trations resulting from approaches along one of the edges.
When this occurred, larvae would stereotypically redirect
movement upon entry at an approximate 45° angle away from
the side bearing vertical stripes, cease movement, and come
to rest on the bottom. Tadpoles would usually return to
Side B within 30-60 seconds. Entries into Side A seldom
surpassed 3-6 cm. Activity within Side A, while proportion-
ately less than that in Side B, occurred away from the
sides of the tank and toward the center of that habitat.

In Test II, behavior varied little in frequency or
patterning from that observed in Test I. However, Group A
tadpoles exhibited much greater tendency to swim along the

sides in Habitat A when tested. Active avoidance of the vertical stripes was not as apparent in this group.

In Test III little stereotypic behavior was noted. Larvae tended to select the uniform white substrate and spent approximately 80-90 percent of their time in very close proximity to the sides of each habitat.

Most tadpoles tested in Test IV evidenced varying stages of limb development and tail resorption. In these stages of late development spontaneous activity greatly decreased in frequency and duration. As a consequence, "avoidance behavior" as described for Tests I and II was uncommon.

Larvae equal to or less than 7 mm tail length clearly reversed their previous avoidance of Side A. Typically, initial movement would occur soon after release and would consist of a direct, rapid movement toward the distant side of Side A. At least 90 percent of all subsequent movement was composed of vertical swimming upward against the sides of Habitat A and passive downward sinking.

Testing of Rana clamitans

Experimentation with Rana clamitans larvae proceded in two directions. Testing for developmental and experiential influences on choice of the standard substrates was virtually unchanged from the procedures used with B. americanus. A second phase of investigation was aimed at the component of the artificial habitat perceived as

"aversive." This required increasing the density within respective rearing tanks to 120 individuals, thus intensifying the possible influence of density-dependent growth inhibition. This added influence should not significantly alter the quality of desired data excepting possible time-developmental interactions.

Testing of Conditioned Substrates: Developmental and Experiential Effects

Four tests using the two standard experimental substrates were completed to elucidate developmental and experiential affects. The general inactivity of these larvae throughout development made it impractical to measure total time spent per side and activity per side. Therefore, the sole indicator of habitat preference was initial choice following release. The adequacy of this as a true indicator was well established in previous testing of R. sylvatica and B. americanus.

Very early preference was tested at days 10-11 posthatching when spontaneous swimming, feeding, and eye development were sufficiently progressed. Subsequent tests were conducted at 25, 52, 66, and 81 days posthatching. Summary of the results and significance of initial choices and prerelease orientation is given in Table 10.

At 10 and 11 days of age all groups exhibited very strong preference of Side B. Excepting Group A, orientation prior to release was also highly significantly directed

Table 10.--Summary of standard test results of R. clamitans showing distribution of initial choices and prerelease orientations and their probabilities.

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Test	Days (Posthatching)	Group	T.L. (mm)	Init. Choice A	Init. Choice B	Probability	Prerelease Orientation A	Prerelease Orientation B	Probability
I	10-11	A	12.0	1	11	< .01	3	9	ns
		B	12.2	1	11	< .01	2	10	< .05
		C	12.4	0	12	< .001	1	11	< .01
II	25-28	A	22.4	3	15	< .01	4	14	< .05
		B	22.0	8	10	ns	6	12	ns
		C	21.9	10	8	ns	10	8	ns
VI	52-54	A	33.7	5	13	< .10	10	8	ns
		B	33.3	5	13	< .10	6	12	ns
		C	33.5	0	11	< .001	5	6	ns
X	66	C	34.6	0	10	< .001	3	7	ns
XIII	81-83	A	40.6	13	7	ns	14	6	ns
		B	42.2	4	16	< .05	7	13	ns
		C	40.2	4	16	< .05	11	9	ns

toward Side B. Behavioral patterns within the test chamber indicating avoidance of Side A were absent. At 25-28 days, Group A followed similar selection patterns in apparent avoidance of its rearing substrate. However, Groups B and C treated substrates nonpreferentially in both first choices and prerelease positioning, indicating a developmental change had occurred in preference in these groups.

Approximately four weeks later (Test VI), results were less definitive. Group C strongly preferred Side B. However, Groups A and B, though tending toward preference of Side B, showed no significant choice and none of the groups indicated significant directional orientation prior to release.

Following Test VI, there remained in conditioning Habitats A and B only enough larvae for one more test. These remaining larvae were subjected to an additional four weeks experience within their rearing habitats. Approximmately midway through this period, ten tadpoles of Group C were tested to determine if any developmental change was affecting habitat choice. No change was evidenced as all tadpoles initially selected Side B.

On days 81-83, results show habitat conditioning apparently affecting substrate choice in Group A. While Group A larvae treated habitats nonpreferentially, Groups B and C significantly selected Side B. The distribution of choices for Group A is particularly noteworthy when compared with choice behavior in the three previous tests.

Investigation of the
Aversive Component

This series of tests were conducted with R. olamitans larvae reared in an alternate control habitat. A summary of the results is given in Table 11. Prerelease orientations were randomly distributed in every case and are not included in the summary.

Test III provided a choice between Habitats B and C, as was carried out in testing of B. americanus tadpoles. No significant selection behavior was demonstrated by any group. However, it should be noted that in each group, over twice as many larvae chose Side C over Side B, suggesting initial choices may not be entirely randomly distributed.

Suggestions by these and prior experiments with R. sylvatica and B. americanus were that the stripes of Side A were in some way aversive. One of the most striking components of this habitat to an observor is the sharp black-white contrast. It was hypothesized that such a contrast when positioned vertically could produce a highly aversive "flicker" across the visual field of a swimming tadpole. This hypothesis was simply tested by replacing the side panels of Side A with horizontal stripes of the same dimensions. Results show that this procedure did not eliminate the aversive quality of that habitat. Only Group B did not strongly avoid this substrate.

Table 11.--Summary of tests investigating "aversiveness" by R. clamitans showing initial choices and their probabilities.

Test	Days (Post-hatching)	Group	T.L. (mm)	Habitats Tested I	Habitats Tested II	Init. Choice I	Init. Choice II	Probability
III	31-32	A	25.7	C	B	12	5	ns
		B	25.2			11	5	ns
		C	24.6			11	5	ns
IV	40-41	A	31.2	Horiz. Striped Sides 1/2:1/2"	B	1	11	< .01
		B	30.6			3	9	ns
		C	30.1			1	11	< .01
V	46-48	A	32.0	Vert. Striped Sides 1/8:1"	C	11	8	ns
		B	32.2			6	13	ns
		C	32.2			8	11	ns
VII	55	C	33.9	Gray Sides	C	9	8	ns
VIII	56	C	32.4	Black Sides	C	8	10	ns
IX	61	C	32.6	Vert. Elodea	C	8	12	ns
XI	69	C	34.4	Vert. Elodea*	C	7	12	ns
XII	76-77	C	36.8	D	C	3	15	< .01
XIV	89	C	43.3	Vert. Striped Sides 1/8:1/2"	C	2	18	< .001
XV	95-96	C	42.2	Vert. Striped Sides 1/4:1"	C	3	17	< .005

*Density doubled.

This subsequently left three major classes of components of the vertical striping to investigate. The pertinent stimulus could involve stripe width and/or frequency, the overall light intensity of the substrate, or the striped pattern itself. Test V retained basic pattern and altered stripe widths to one-eighth inch black and one inch white thus changing not only the ratio of black to white, but also the overall reflective intensity of the substrate. In view of the possible confounding influence of the base stripes of Side B, in this and subsequent tests novel substrates were tested against a uniform white substrate. None of the groups demonstrated active differentiation of these substrate types.

In consideration of these findings, it would be an easy task to test if the change in overall substrate intensity in Test V was responsible for these behaviors. To accomplish this, sidepanels of Side A were removed exposing the gray tank sides. This closely approximated the overall intensity produced by the standard substrate. Group C tadpoles behaved nonpreferentially in this situation and when presented with a habitat of uniform black replacing the gray in Test VIII, likewise responded indifferently. This indicated intensity alone was not a controlling influence but could still be an important interaction with another component.

In a study by Wiens (1970) with <u>Rana</u> <u>aurora</u> tadpoles, it was suggested that the striped habitat selected

by striped-conditioned tadpoles may have been analogous to linear vegetation found in the natural habitat of this species. Langdon (1971) concluded that fiddler crabs, Uca pugilator, approached vertical striping because of its resemblance to protective grasses and other vegetation in their habitat. On the same premise, a test was designed to test the possible single influence of a natural occurring linear plant on selection of a habitat by tadpoles. On two-thirds of one side of an all white test tank, vertical segments of Elodea canadensis were distributed evenly. Individuals were released approximately one-third of the way into the featureless side of the tank. In Test XI the same basic procedure was followed but the density of Elodea within Side I was roughly doubled. In neither case did the presence of Elodea nor its density have any significant influence on selection of a habitat.

A typical pond of southern Michigan differs little in obvious visual features. One predominant variable factor from an observor's standpoint, is the structural type and density characteristics of aquatic vegetation. A simple breakdown of vegetation type according to structure might be those species of a pinnate or branched structure (like Elodea) as opposed to plants arranged in linear uniformity. In the present case, tadpoles avoid vertical, artificial striping that is highly uniform, but do not react to actual vegetation of a pinnate nature. In Test XII, Side A was modified to test this basic difference in patterning. The

uniformity of type A substrate was disrupted by super-imposing horizontal "fringe" on the black stripes (Figure 2). This "fringe" component, however, was ineffective in modifying avoidance behavior when tested against an all white habitat. Only three of 18 larvae initially selected Side D.

Further experimentation with patterns and new variables was impractical with the remaining number of larvae. The best direction seemed to be with varying relative amounts of black and white stripes without changing basic pattern. In Test V, alteration of stripe widths and reducing the black to white ratio to 1:8 resulted in no preference. Because overall reflective intensity is not a dominating influence, Test V results suggest tadpoles were responding to width or frequency of either the black or white stripes or to some relationship between the two stripes in which a ratio is the important feature. In Test XIV, it was decided to leave unchanged the width of the black stripe (one-eighth inch) but to reduce the white stripe width to that of the standard substrate (one-half inch). If this habitat was treated nonpreferentially, indications would be that either the black width or ratio of black to white was important. If avoided, ratio may still be important but in addition could mean the width of the white stripe was the recognized feature. Results showed avoidance was highly significant with only two of 20 Group C larvae selecting that habitat.
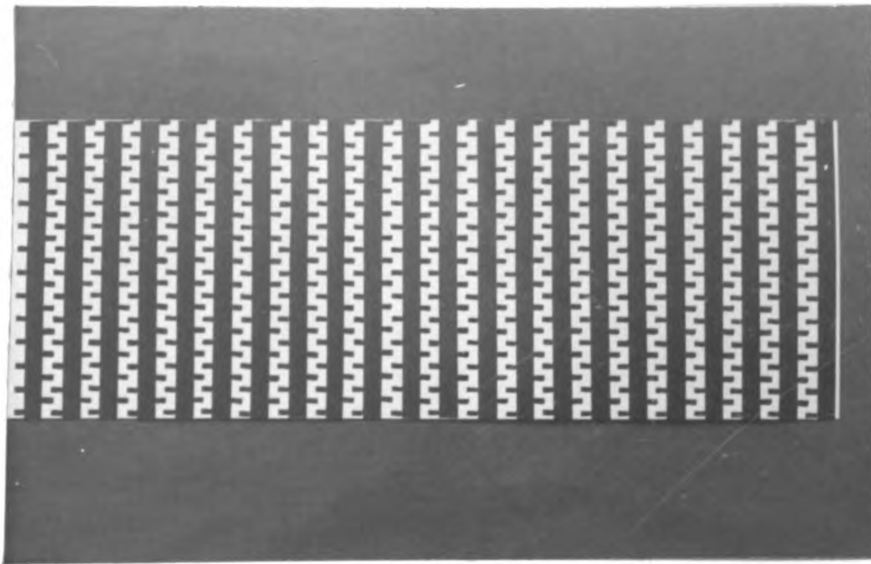
Figure 2.   Test substrate used in Habitat D.

A final test (Test XV) was designed to elucidate
the importance of either ratio or white stripe width in
aversiveness. The avoided ratio of 1:4 was retained while
enlarging the white stripe to that employed in Test V (one-
fourth inch black:one inch white). Definite avoidance was
again demonstrated, strongly suggesting that the ratio of
black to white is important in illiciting avoidance be-
havior in these larvae.

DISCUSSION

Developmental Affects

At two instances, results showed habitat choice
behavior was strongly influenced by developmental stage. In
testing of <u>Bufo</u> <u>americanus</u> tadpoles, selection of experi-
mental substrates was based on an inherent aversion to
Habitat A (striped sides). Prior habitat experience had
no apparent influence. Preference for Habitat B by all
groups persisted throughout development until larvae prog-
ressed into late metamorphic transformation. On days 34-37
posthatching, those larvae of Groups B' and C' with tails
resorbed to 7 mm or less reversed previous preference and
significantly selected Habitat A (Table 1).

At this stage, larvae have ceased feeding and
activity is greatly diminished from earlies stages. Tad-
poles appear frog-like with fore- and hind-limbs well
developed. Metamorphosis is progressing very rapidly with
almost complete tail resorption from 7 mm and conversion to
terrestrial life occurring within approximately 24 hours at
laboratory temperatures. There is also evidence of a
reversal in y-axis orientation occurring in tadpoles just
prior to emergence. McKeown (1968) found that larvae of

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B. woodhousii and R. catesbeiana originally orient toward
deep water but show shoreward preferences prior to emer-
gence. A similar reversal just prior to emergence in bull-
frogs was recorded by Goodyear and Altig (1971). During
this period it is critical that they climb to a dry surface
and resume terrestrial feeding or drowning will result.

One would hypothesize that the mechanism for
accomplishing this end would require strong and inherent
attending to cues such as decreasing water depth and emer-
gent and submergent vegetation and possibly sun compass
orientation. Such a mechanism would primarily be based on
visual and tactile cues. Indeed, Brockelman (1969) con-
sidered B. americanus larvae to be transformed when "they
showed a decided preference for solid substratum above the
water surface and when the tail was shorter than the hind
legs." This description would place tadpoles in the approx-
imate stage reversal of preference occurred. It is likely
that the dark mid-water stripes of Habitat A resemble a
"climbable surface" to the transforming larvae. Behavior
within the test chamber supports this contention.

Larvae with a tail length of 7 mm or less ex-
hibited stereotypic behavior following release. Upon
release, active choice was initiated usually within the
first one to two minutes. This almost invariably consisted
of direct swimming perpendicular from the border to the
extreme side of Habitat A. Once actual contact with the
side was made, subsequent swimming was nearly exclusively

in the vertical plane. Seldom was downward movement actively directed, but rather consisted of passive sinking.

A second observation indicating a developmental influence occurred with R. clamitans larvae of 25-28 days of age at Test II (Table 10). A previous test at 10-11 days of age demonstrated strong preference of Habitat B by all groups in both prerelease orientation and first choice. At Test II, Group A continued to clearly prefer Habitat B in both categories over its conditioning substrate. However, Groups B and C were randomly distributed in their initial choices and prerelease orientations. Furthermore, the change to nonpreference exhibited by Groups B and C was transient in that both groups avoided Habitat A in later tests. This does not seem to represent a procedurally induced phenomenon as Group A tadpoles were tested at the same time and subject to identical experimental and rearing conditions. The functional or possible adaptive significance of such a change in behavior is difficult to perceive. There occurs no known change in morphology (except size), feeding habits, or any other aspect of larval life that may be critical to larvae and that might correspond with the time period. Without more specific information about the larval ecology of this species it is impossible to interpret this lack of preference. Repetition of this phase of the experiment would be highly desirable.

Experiential Affects

Rearing experience had no affect on habitat choice in either R. sylvatica tadpoles or during any period in the larval life (34-37 days) of B. americanus. (Unfortunately, the time to metamorphosis in the later group was shortened due to an increase in laboratory temperature.)

Two affects attributable to habitat conditioning were both observed in standard testing of R. clamitans larvae. On days 25-28 posthatching, green frog tadpoles of Group A continued to avoid their rearing substrate while Groups B and C showed no preference. Prior experience within conditioning Habitat A by Group A was sufficient to override a more general developmental change occurring in Groups B and C. No explanation for this developmental change is readily apparent from available data. It is also difficult to determine if there is a true advantage to this experiential influence. There seems to be no situation where benefit could be derived during this period of development short of limited and transient spatial segregation due to differential experiences.

Experientially modified behavior was also evidenced late in the testing of R. clamitans Group A tadpoles. Standard tests initiated at 10 and 25 days of age indicated typical strong avoidance of its rearing substrate. At 52 days only 5 of 18 selected Habitat A (not significant at p = .05). However, at days 81-83, 13 of 20 larvae tested selected the conditioning substrate (A) while Groups B

and C significantly avoided that substrate. This seems to represent a period in which acceptance, or at least acclimation, to the conditioned substrate is taking place. This hardly seems an "adaptive" change occurring this late in larval life and more probably is simply an habituation to an inherently aversive stimulus.

Prior experience of B. americanus larvae had a limited influence on behavior within the test chamber. In Tests I and II movements by all groups illustrated a strong avoidance to the vertical stripes of Side A. However, in Test II, Group A larvae exhibited a much greater tendency to swim along the sides of Side A than the other groups that were previously unexposed to that substrate. Exposure to Habitat A within the first three weeks of larval life was sufficient to decrease spontaneous reaction to the vertical stripes but not sufficient to abolish avoidance of that habitat.

Selection Based on Avoidance

Responses to standard substrates were relatively stereotypic in choice and behavior in the three species tested. Habitat B with the striped bottom was nearly invariably preferred over the striped sides of Habitat A. Conditioning had little affect on modifying choice. The primary motivator of this response seemed one of avoidance to the vertical striping of Habitat A, rather than positive

orienting to Habitat B. Evidence for this is reflected strongly in activity patterns within the test tank.

It was commonly observed in testing of R. sylvatica and B. americanus that when Side A was approached from Side B, tadpoles would rapidly reverse direction upon reaching the border. If Side A was penetrated, distance of penetration was very short, and movement, particularly by B. americanus, was directed sharply away from the striped sides upon entry. Whereas time spent in Side B was almost exclusively along the edges, time spent in Side A was toward the center of that habitat away from the stripes. When given a choice between Habitat B and an all white control habitat (C), B. americanus and R. clamitans tended to prefer Side C. This supports the contention that the vertical stripes of Side A are the avoided component and that Side B was in fact discriminated. Results also suggest that the bottom stripes of Side B may be aversive but to a lesser degree.

Spontaneous avoidance behavior of the vertical stripes of Side A was not exhibited by larvae of R. clamitans, but initial choices generally showed preference for Side B. Evidence indicated that a similar selective process (avoidance) was operating in this species as the previous two, and several tests were designed to deduce the specific component(s) of Habitat A that was aversive (Table 11).

The possibility that aversive visual flicker was responsible for avoidance of Habitat A was tested by allowing tadpoles to choose between Habitat B and a habitat bearing horizontal stripes in place of the vertical stripes. No difference in behavior was noted as this substrate was similarly avoided. This generally agrees with Munn's (1940) unsuccessful efforts at teaching Hyla tadpoles to discriminate between vertical and horizontal stripes. Overall reflective intensity, as tested with uniform gray and black sides against all white substrates, was in itself not a factor influencing selection. Pattern disruption facilitated by addition of a fringed component (Habitat D) to the standard "A" substrate did not change its aversive quality as evidenced by distribution of initial choices.

The only significant change in choice behavior resulted from manipulation of relative proportions of vertical black and white stripes. Retention of basic pattern and changing black stripe widths to one-eighth inch and white stripe widths to one inch resulted in no preference when tested against an all white substrate. Subsequent tests of striping sought to resolve the components effective in apparent "neutralization" of the aversive quality of Habitat A. A second test doubled the nonpreferred stripe ratio from 1:8 to 1:4 but retained the one-eighth inch black stripe (one-eighth inch black, one-half inch white). Avoidance was restored as Group C larvae strongly selected the uniform white substrate.

These results suggested that either the single effect of the white stripe width or the actual black-white ratio was the feature affecting habitat selection. A final test retaining the avoided 1:4 ratio while employing one inch wide white intervals was designed to test these factors. Avoidance of this substrate by Group C was highly significant. Apparently the ratio of the contrasting black to white 'stripes is the aversive component affecting observed selection in this species.

A number of problems arise in attempting to interpret or assign adaptive function to the described responses. The experimental visual parameters were highly artificial in design. Ecological interactions and microdistribution of anuran larvae are virtually uninvestigated. Sources of mortality and the degree to which certain factors affect development lack quantification. Furthermore, with the exception of limited work with adults (Maturana et al., 1960; Lettvin, et al., 1959; and Ewert, 1974) visual discrimination and pattern resolution capabilities of anuran larvae are unknown. In consequence, assumptions regarding direct application of the described avoidance behavior to a natural situation are purely speculative and have little substantive basis.

The strength of the aversive stimulus, its persistence in development, and its relative specificity in three species of anurans demonstrate a strong hereditary basis and suggest the behavior is being acted upon

selectively. For selection to be taking place, one must assume a situation exemplifying similar qualities of dark-light ratios currently exists within the natural distribution of these animals and that avoidance of this feature has a profound influence on some aspect of larval life (e.g., in predatory or competitive interaction).

Direct predator resemblance is unlikely. The striped pattern is not like any primary predator and tadpoles show little reaction to visual "threats" around them unless they are accompanied by actual mechanical disturbance. One of the few characteristics of southern Michigan ponds approaching resemblance to the artificial components is submergent vegetation type and/or density. Vegetation type has been shown to be important in the selection of a stream habitat by sticklebacks (Baker, 1971; and Hagen, 1967). Wiens (1970) thought that the stripes selected by conditioned Rana aurora tadpoles may have resembled linear vegetation of their natural habitat. Longdon (1971) drew similar conclusions in his study of the fiddler crab, Uca pugilator. Avoidance on this basis to specific vegetation type or density could be important to anuran larvae from a competitive or predatory standpoint. However. the important element of contrast seems to be lacking in the submerged aquatic environment and there is no evidence indicating spatial segregation correlating with vegetation in the literature or from personal observation. Indeed, R. clamitans larvae failed to actively discriminate two

densities of the common _Elodea_ _canadensis_ when tested
against uniform white substrates.

The only conceivable situation where visual cues of
high contrast and varying density could be perceived by
tadpoles is that of overhead emergent vegetation, or even
terrestrial vegetation, outlined against the sky. These
cues would in fact be within the visual field of these tad-
poles in respect to the dorsal positioning of the eyes and
could be instrumental in y-axis orientation.

An alternative application that warrants consider-
ation when working with amphibian larvae is the possibility
of relevance of these cues to adults. Unfortunately,
retention of learned or inherent behaviors beyond meta-
morphosis is virtually uninvestigated for amphibians. But
because of the drastic change of habitat from completely
aquatic to semi-terrestrial, it is possible that a function-
ing behavior of the type described could be present in the
larval stage without adversely affecting their survival and
come into use when the animal transforms into the terres-
trial stage. In juveniles and adults, overhead cues of the
type postulated above could become instrumental in short
range orientation toward bodies of water. Overhead
vegetation of high density might be avoided thus "directing"
movements toward more open areas where water might occur.
The more open areas would be unavoided or oriented toward.
Although such a mechanism alone would likely be of little

use, when used in conjunction with other recepted cues (e.g., sun compass orientation) it could become important.

Ewert (1974) studied the neurophysiological basis of orienting and avoidance responses to small objects in adult Bufo bufo. Behavioral response was dependent on the object size, configuration, its angular velocity, and its contrast with the background. There may be some relationship between aspects of this visually guided behavior in adults to pertinent visual stimuli in the larval stage. Further investigation into this area is needed.

In view of the present knowledge concerning general ecology of anuran tadpoles, there is no clear "functional" explanation for the observed tadpole selection behavior. The great difficulty lies in defining an environmental parameter that reflects visually the recognized components of the imposed experimental habitat. However, the observed components may in fact be incomplete in representation of the entire relevant stimulus or may even represent a super-normal stimulus further confounding analysis. Perhaps the best approach would be a neurophysiological breakdown of a particular response (Lettvin, et al., 1959) and its direct relationship to an innate behavior (Ewert, 1974). In any case, if an adaptive function is sought, determination must be made of the microdistributional relationships of anuran larvae and the factors influencing survival.

Activity Over Test Substrates

As an additional behavioral measure, the total time spent active over respective test substrates was recorded for R. sylvatica and B. americanus tadpoles. The extremely low level of activity of R. clamitans larvae prevented accurate assessment of this relation with habitat in this species.

The time an animal is active within particular habitats has been interpreted as an indication of the quality of that habitat as perceived by the animal. Sale (1969b) based much of his theory of a "selection mechanism" on the level of activity or "appetitive exploration" exhibited by an animal in one particular habitat-type. Sale postulates a negative feedback system whereby under adequate environmental conditions feedback intensity to the "selection mechanism" will lower the appetitive exploration. Magnitude of activity increases in more inadequate situations. He tested his theory using the surgeon fish, Acanthurus triostegus, and recorded behavior corroborating predictions based on the habitat selection mechanism.

Few researchers other than Sale have examined activity as a component of habitat selection. However, Harris (1952) and Wecker (1963) cite evidence that the prairie deermouse, Peromyscus maniculatus is generally more active in its preferred habitat. Quertermus (1972) found no consistent correlation of activity with habitat preference in his study of Tilapia mossambica.

In the present study, R. sylvatica tadpoles spent approximately equal amounts of time active over each test substrate. B. americanus, however, spent a great deal more time active over the preferred substrate (Table 1). According to Sale's model, one would predict greater activity in nonpreferred Habitat A. However, other factors warrant consideration. Activity within Habitat B was concentrated almost entirely around the sides of that habitat. Also random observation of activity within rearing tanks indicated movement to be primarily around the edges. Within the test tank stripes appear to be avoided by the tadpoles regardless of their positioning. Assuming this to be true, the comparatively high activity levels while over test Habitat B could result from the interaction of two factors. The apparent tendency to move along edges of an enclosure combined with an attempt to avoid the bottom striping of Habitat B could be capable of increasing considerably total activity over that substrate. In Habitat A, tadpoles strongly avoid side striping, thus displacing them away from the edges of that habitat. Assuming interaction with a vertical edge functions to "stimulate" activity, total movement in this habitat would substantially lower. If such an interaction is operating in this situation, Sale's model has not been refuted.

It is also conceivable that following the typical initial choice of Habitat B, subsequent entry into highly

aversive Habitat A is sufficiently startling or "shocking"
to cause the observed immediate ceasing of movement. If
this is the case, direct comparison with Sale's results is
not possible. Sale's experiment measured only random move-
ments within a single habitat and would not account for
behavior within a highly aversive situation which allows a
choice between two habitats. The movements of tadpoles in
the test bank could not be classified as random or "ex-
ploratory" in terms of Sale's criteria.

Relative Importance of Midwater and Bottom Stripes

Standard experimental substrates were designed to
test the relative importance of midwater and bottom cues in
selection of the test substrates. Because both habitats
utilized identical visual components, selection would
essentially reflect differences in positioning of stripes.
All species tested generally preferred Habitat B (bottom
stripes) to Habitat A (side stripes). However, the stronger
stimulus does not seem to be a positive attraction to
Habitat B, but rather avoidance of the vertical striping of
Habitat A as previously discussed.

Tadpoles discriminate each substrate and aversive-
ness between the two substrates apparently differs only in
degree. When allowed to choose between an all white sub-
strate and Side B, B. americanus and R. clamitans larvae
tend to prefer the featureless substrate. Stimulus strength

and quality is likely affected in two ways. From the view-
point of a tadpole, the striping of Habitat B would present
less effective surface area impinging on the retina than
the vertical striping of Habitat A because of its bottom
positioning. Also because of positioning, stripes would
likely lack the distinctness of Habitat A and may appear
highly blurred to a tadpole sitting or moving along the
bottom.

A second consideration is the effective visual field
of the tadpoles. Eye placement in these species is dorsal.
Thus, the perceptual field of bottom cues is decidedly
decreased, due partly to the lateral bulge of the body
ventral to the eyes. Eye positioning seems related to their
bottom dwelling habits. Heyer (1972) observed that of the
species of frogs studied in Thailand, midwater and surface
forms generally had laterally directed eyes while bottom
dwellers possessed dorsally directed eyes. This seems to
indicate the greater relative importance of midwater and
overhead cues to these species by virtue of their bottom
habits and effective visual field. In the present study,
midwater stripes provided the greatest stimulus in selection
of a habitat.

SUMMARY

1. Three species of anuran larvae were reared in
    artificial habitats that varied only in visual
    appearance. At different points in development,
    samples of individuals from each habitat were
    allowed to choose between two substrate types to
    determine the influence of developmental stage and
    prior experience on habitat selection. The design
    of the standard test substrates allowed discernment
    of the relative importance of midwater and bottom
    cues in selection of a habitat. Results showed
    highly stereotyped behavior dependent primarily on
    cues situated in the vertical plane (midwater).

2. In a preliminary study, larvae of the wood frog,
    Rana sylvatica, were reared from the hatchling
    stage in two experimental habitats. Habitat A was
    composed of alternating vertical black and white
    stripes of one-half inch widths affixed to the
    sides and a uniform white bottom. Habitat B con-
    sisted of identical black and white striping on the
    bottom and uniform white sides. Larvae were

presented with a choice between these two con-
ditioning habitats after 20-25 days experience.

3. Larvae conditioned in Habitat A (Group A) signifi-
cantly demonstrated preference for Habitat B in
both initial choice and total time. Of nine larvae
tested from Habitat B (Group B), no preference was
evidenced by initial choice, however, total time
indicated a strong preference for Habitat B. During
testing, spontaneous reactions by individuals of
both groups when Habitat A was approached, indicated
that this selection was based on an avoidance to the
vertical stripes of that habitat.

4. Larvae of the American toad, Bufo americanus, were
reared from hatchlings through metamorphosis in
experimental Habitats A and B and a uniform white
control habitat (Habitat C). When allowed to choose
between Habitats A and B on days 9-11 and 23-25
posthatching, all three groups demonstrated prefer-
ence for Habitat B in initial choice and total
time. Swimming behavior within the test apparatus
during each test indicated strong avoidance to the
vertical stripes of Habitat A. Activity for every
group was much greater while over preferred
Habitat B. This relationship of activity and
habitat is discussed in relation to other studies.

5. During the final standard test on days 34-37 post-hatching, tadpoles were in varying stages of transformation. Tadpoles of Groups B and C in early metamorphosis with tail lengths greater than 7 mm continued to strongly avoid Habitat A in initial choice and total time. Those animals with tails resorbed to 7 mm or less reversed choice behavior and strongly selected Habitat A. This pronounced developmental change occurs at a time when larvae must soon leave the water if they are to survive and seems to represent strong attending to cues resembling a climbable surface (vertical stripes). This is substantiated by swimming behavior and orientation within Habitat A during testing.

6. On days 32-33 larvae were presented with a choice between Habitat B and control Habitat C. Groups tended toward preference of Habitat C. This substantiated indications that the side striping of Habitat A was the avoided component and suggested that the bottom striping was not only discriminated, but was also aversive in a lesser degree.

7. Larvae of the green frog, <u>Rana</u> <u>clamitans</u>, were reared from hatching in the three standard conditioning habitats and tested for preference through development. Because of very low levels of spontaneous activity, the sole indicator of habitat selection was initial choice. On days 10-11, all

groups significantly chose Habitat B and also demonstrated orientation toward that habitat immediately prior to release. Group A tadpoles continued to demonstrate preference for Habitat B on days 25-28 in both initial choice and prerelease orientation. However, Groups B and C exhibited no preference to the standard substrates. It appears that a general developmental change in preference occurred at this stage and prior exposure of Group A to Habitat A was sufficient to overcome this change. There is no apparent explanation for this developmentally influenced behavior. On days 52-54, Groups A and B tended toward selection of Habitat B, but not significantly. Group C strongly preferred Habitat B in this test and again on day 66. The final standard test on days 81-83 posthatching, showed Groups B and C avoiding Habitat A while Group A indicated an influence on selection attributable to prior conditioning by showing no preference.

8. Between standard tests with R. clamitans, a variety of novel substrates, substituted in place of Habitat A, were tested in an effort to determine the "aversive" components of the vertical stripes. Aversive visual flicker was eliminated as a factor by testing with horizontal stripes. Overall reflective intensity of the substrate was likewise

discarded as a single influencer of selection by
testing uniform gray and uniform black sides. Tad-
poles did not actively discriminate vertical seg-
ments of aquatic vegetation (<u>Elodea</u> <u>canadensis</u>)
and when horizontal "fringe" was added to the black
stripes of Habitat A, tadpoles continued to strongly
avoid that substrate. By manipulation of widths
and ratios of the vertical black and white stripes,
it was concluded that the ratio of the contrasting
stripes was important in eliciting avoidance to
Habitat A in this species. Adaptive implications
of these behaviors are discussed.

LITERATURE CITED

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Altig, R. and E. D. Brodie. 1972. Laboratory behavior of
    Ascaphus truei tadpoles. J. Herpetol. 6(1):21-24.

Anderson, P. K. 1954. Studies in the ecology of the narrow-
    mouthed toad, Microhyla carolinensis carolinensis.
    Tulane Stud. Zool. 2(2):15-46.

Baker, M. C. 1971. Habitat selection in fourspine stickle-
    backs (Apeltes quadrasus). Amer. Midl. Nat. 85(1):
    239-242.

Baldes, R. J. and R. E. Vincent. 1969. Physical parameters
    of microhabitats occupied by brown trout in an
    experimental flume. Trans. Amer. Fish. Soc. 98(2):
    230-238.

Bragg, A. N. 1940a. Observations on the ecology and
    natural history of Anura I. Habits, habitat and
    breeding of Bufo cognatus Say. Amer. Nat. 74
    (753,754):322-349 and 424-438.

Bragg, A. N. 1940b. Observations on the ecology and
    natural history of Anura II. Habits, habitat, and
    breeding of Bufo woodhousii woodhousii. Amer.
    Midl. Nat. 24(2):306-321.

Bragg, A. N. 1943. Observations on the ecology and
    natural history of Anura XV. The hylids and
    microhylids in Oklahoma. Great Basin Nat. 4:62-80.

Bragg, A. N. and C. C. Smith. 1942. Observations of the
    ecology and natural history of Anura IX. Notes on
    breeding behavior in Oklahoma. Great Basin Nat.
    3:33-50.

Brattstrom, B. H. 1962. Thermal control of aggregation
    behavior in tadpoles. Herpetologica 18(1):38-46.

Brockelman, W. Y. 1969. An analysis of density effects
     and predation in Bufo americanus tadpoles. Ecology
     50(4):632-644.

Brown, L. E. and J. R. Pierce. 1965. Observations on the
     breeding behavior of certain anuran amphibians.
     Texas J. Sci. 17(3):313-317.

Butler, R. L. and V. M. Hawthorne. 1968. The reactions of
     dominant trout to changes in overhead artificial
     cover. Trans. Amer. Fish. Soc. 97(1):37-41.

Ewert, J. P. 1974. The neural basis of visually guided
     behavior. Sci. Amer. 230(3):34-42.

Goodyear, C. P. and R. Altig. 1971. Orientation of bull-
     frogs (Rana catesbeiana) during metamorphosis.
     Copeia 1971(2):362-364.

Gosner, K. L. 1960. A simplified table for staging anuran
     embryos and larvae with notes on identification.
     Herpetologica 16(3):183-190.

Hagen, D. W. 1967. Isolating mechanisms in threespine
     sticklebacks (Gasterosteous). J. Fish. Res. Bd.
     Canada 24(8):1637-1692.

Haines, T. A. and R. L. Butler. 1969. Responses of year-
     ling smallmouth bass (Micropterus dolomieui) to
     artificial shelter in a stream aquarium. J. Fish.
     Res. Bd. Canada 26(1):21-31.

Harris, V. T. 1952. An experimental study of habitat
     selection by prairie and forest races of the deer-
     mouse, Peromyscus maniculatus. Contr. Lab. Vert.
     Biol. Univ. Michigan 56:1-53.

Heatwole, H. 1961. Habitat selection and activity of the
     wood frog, Rana sylvatica Le Conte. Amer. Midl.
     Nat. 66(2):301-313.

Heatwole, H. 1962. Environmental factors influencing
     local distribution and activity of the salamander,
     Plethodon cinereus. Ecology 43(3):460-472.

Herreid, C. F. and S. Kinney. 1967. Temperature and
     development of the wood frog, Rana sylvatica, in
     Alaska. Ecology 48(4):579-590.

Heyer, W. R. 1973. Ecological interactions of frog larvae
     at a seasonal tropical location in Thailand. J.
     Herpetol. 7(4):337-361.

Hilden, O. 1965. Habitat selection in birds. Ann. Zool. Fennici 2:53-75.

Hunsaker, D., II and R. W. Crawford. 1964. Preferential spawning behavior of the largemouth bass, Micropterus salmoides. Copeia 1964(1):240-241.

Hutchison, V. H. 1958. The distribution and ecology of the cave salamander, Eurycea lucifuga. Ecol. Monogr. 28(1):1-20.

Klopfer, P. H. 1963. Behavioral aspects of habitat selection: the role of early experience. Wilson Bull. 75(1):15-22.

Klopfer, P. H. 1965. Behavioral aspects of habitat selection: a preliminary report on stereotypy in foliage preferences of birds. Wilson Bull. 77(4): 376-381.

Klopfer, P. H. 1969. Habitats and territories. Basic Books, Inc., New York. 117 p.

Klopfer, P. H. and J. P. Hailman. 1965. Habitat selection in birds. In: Advances in the study of behavior. Lehrman, D. S., R. A. Hinde, and E. Shaw [eds.]. Academic Press, N.Y. Vol. I, pp. 279-303.

Kwain, W. and H. R. McCrimmon. 1967. The behavior and bottom color selection of the rainbow trout, Salmo gairdneri Richardson, exposed to different light intensities. Anim. Behav. 15(1):75-78.

Lack, D. 1933. Habitat selection in birds with special references to the effects of afforestation on the Breckland avifauna. J. Anim. Ecol. 2:239-262.

Langdon, J. W. 1971. Shape discrimination and learning in the fiddler crab Uca pugilator. Ph.D. Thesis, Florida State University.

Lettvin, J. Y., H. R. Maturana, W. S. McCulloch, and W. H. Pitts. 1959. What the frog's eye tells the frog's brain. Proc. Inst. Radio. Engrs., N.Y. 47:1940-1951.

Lucas, E. A. and W. A. Reynolds. 1967. Temperature selection by amphibian larvae. Physiol. Zool. 40:(2):159-171.

Maturana, H. R., J. Y. Lettvin, W. S. McCulloch, and W. H. Pitts. 1960. Anatomy and physiology of vision in the frog (Rana pipiens). J. Gen. Physiol. 43(6): 129-176.

McCrimmon, H. and W. Kwain. 1966. Use of overhead cover by rainbow trout exposed to a series of light intensities. J. Fish. Res. Bd. Canada 23(7):983-990.

McKenzie, D. S. and R. M. Storm. 1970. Patterns of habitat selection in the clouded salamander, Aneides ferreus (Cope). Herpetologica 26(4):450-454.

McKeown, J. P. 1968. The ontogenetic development of y-axis orientation in four species of anurans. Ph.D. Thesis, Mississippi State University.

Mullally, D. P. 1953. Observations on the ecology of the toad Bufo canorus. Copeia 1953(3):182-183.

Munn, N. L. 1940. Learning experiments with larval frogs. J. Comp. Physiol. Psychol. 29:97-108.

Pache, J. 1932. Formensehen bei Froschen. Zeit. Vergl. Physiol. 17:423-463.

Quertermus, C. J. 1972. Prior experience as a factor in habitat selection of the cichlid fish Tilapia mossambica. Ph.D. Thesis, Michigan State University.

Ralin, D. B. 1968. Ecological and reproductive differentiation in the cryptic species of the Hyla versicolor complex (Hylidae). Southwest. Nat. 13(3):283-300.

Sale, P. F. 1968. Influence of cover availability on depth preference of the juvenile manini, Acanthurus triostegus sandvicensis. Copeia 1968(4):802-807.

Sale, P. F. 1969a. Pertinent stimuli for habitat selection by the juvenile manini, Acanthurus triostegus sandvicensis. Ecology 50(4):616-623.

Sale, P. F. 1969b. A suggested mechanism for habitat selection by the juvenile manini Acanthurus triostegus sandvicensis Streets. Behaviour 35: 27-44.

Sale, P. F. 1971. Apparent effect of prior experience on a habitat preference exhibited by the reef fish, Dascyllus aruanus (Pisces: Pomacentridae). Anim. Behav. 19(2):251-256.

Sale, P. F. 1972. Influence of corals in the dispersion of the pomacentrid fish, Dascyllus aruanus. Ecology 53(4):741-744.

Sargent, T. D. 1965. The role of early experience in the nest building of the zebra finch. Auk 82(1):48-61.

Savage, R. M. 1952. Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. Lond. 122:467-514.

Sexton, O. and E. P. Ortleb. 1966. Some cues used by the leptodactylid frog, Engystomops pustulosus, in selection of the oviposition site. Copeia 1966(2): 225-230.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco.

Svardson, G. 1949. Competition and habitat selection in birds. Oikos 1:157-174.

Thoms, C. L. 1974. Effects of prior experience upon nesting and resting site selection by the Mossambique mouth brooder, Tilapia mossambica. Ph.D. Thesis, Michigan State University.

Vlaming, V. L. de and R. B. Bury. 1970. Thermal selection in tadpoles of the tailed-frog, Ascaphus truei. J. Herpetol. 4(3-4):179-189.

Wecker, S. C. 1963. The role of early experience in habitat selection by the prairie deer mouse, Peromyscus maniculatus bairdi. Ecol. Monogr. 33(4): 307-325.

Wells, M. M. 1924. Chrophilus nigritus. The swamp tree frog. Turtox News 2:22.

Wiens, J. A. 1970. Effects of early experience on substrate pattern selection in Rana aurora tadpoles. Copeia 1970(3):543-548.

Wiens, J. A. 1972. Anuran habitat selection: early experience and substrate selection in Rana cascadae tadpoles. Anim. Behav. 20(2):218-220.

Wilbur, H. M. 1972. Competition, predation, and the structure of the Ambystoma-Rana sylvatica community. Ecology 53(1):3-21.

Wilbur, H. W. and J. P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182(4119): 1305-1314.

Workman, G. and K. C. Fischer. 1941. Temperature selection and the effect of temperature on movement in frog tadpoles. Amer. J. Physiol. 133:P499-P500.