

AN ABSTRACT OF THE THESIS OF

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Environmental Cues, Ontogeny, and Adaptive Significance  
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Dr. Robert M. Storm

Habitat selection behavior was investigated as a proximate mechanism influencing spatial distributions of three species of anuran larvae. Rana cascadae, Hyla regilla, and Bufo boreas overlap in distribution in lakes and ponds of the Cascade Mts. and were chosen to provide a comparative basis for analyzing habitat selection behavior. Major objectives of the study were to define the proximate cues used by tadpoles in selecting a habitat, describe the ontogenetic basis of cue preference, and delineate the possible selective constraints acting on the habitat selection process.

At one study site (Little Three Creek Lake), tadpole distributions were quantitatively assessed relative to habitat variables (shore distance, temperature, dissolved oxygen, depth, substrate type, and macro-vegetation) throughout a summer. These data were subjected to correlation analysis and multiple linear regression analysis. Significant associations for each species were with temperature, depth,

substrate, vegetational patterns, and shore distance. Tadpoles were most frequently found in protected areas along shallow gently sloping shorelines, over fine as opposed to coarse substrate types, in high temperature areas, and often amongst vegetation (Carex and Isoetes) (observations consistent at over 25 other sites surveyed). Larvae of each species selected similar habitat types (overlap was high) but B. boreas was more of habitat generalist due to high dispersal tendencies, schooling behavior, and mid-water feeding habits. R. cascadae and H. regilla larvae tended to remain in the vicinity of the oviposition sites which were sometimes patchily distributed. Mark and census experiments showed that dispersal was limited in these species, certain features (e.g. substrate type, deep or cold water) represented barriers to dispersal, tadpoles tended to disperse more from nonpreferred habitats than preferred habitats, and disappearance rates were greater in nonpreferred habitats. Benthic sample analyses showed "preferred" substrates (fine particulates) contained greater amounts of total organics and chlorophyll (i.e., potential food sources) than coarse substrates which were avoided by tadpoles.

Because such field studies do not distinguish correlated variables from those actually perceived and responded to by tadpoles, laboratory choice experiments were used. Tadpoles were reared over one of four substrates (smooth substrate, sand, gravel, or rock) and periodically tested for preferences during development. Larvae of each species (lab-reared and wild tadpoles) preferred finer grained substrates regardless of rearing experience, age, or population source tested. Tadpoles preferred an open habitat to one with artificial vegetation that

resembled Carex and all species exhibited a strong tendency to aggregate with conspecifics when tested in the laboratory. R. cascadae tadpoles preferred water depths at which they were reared and wild B. boreas larvae tended to prefer shallow depths. Stereotypic responses of R. cascadae were modified following rearing in a complex environment where a positive stimulus (high temperature or food) was associated with a nonpreferred substrate, and a "negative" stimulus (lower temperature or no food) was associated with a preferred substrate. Tadpoles came to prefer the previously nonpreferred substrate when later tested but these modified preferences were gradually lost when tadpoles were removed from the rearing regime. With the exception of vegetation preference tests, lab choice experiments corroborated field observations.

Habitat selection seems to be a complex process in these tadpoles. More than one or two key cues are involved, cues responded to are of a structural, microclimatic, and biologic nature, responses to some cues were very rigid and to others modifiable by experience, and some adaptability in behavioral responses was evident. In the ephemeral breeding habitats of the high Cascades, traits that influence growth are likely to be closely linked to overall fitness. The habitat selection behaviors observed in these larvae (i.e., habitats and factors conducive to rapid growth) reflect an overall strategy to minimize the time needed for successful completion or metamorphosis by maximizing components of growth and development.

Habitat Selection Behavior in Three Species of Anuran Larvae:  
Environmental Cues, Ontogeny, and Adaptive Significance

by

Richard K. O'Hara

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HABITAT SELECTION BEHAVIOR IN THREE SPECIES OF ANURAN LARVAE:  
ENVIRONMENTAL CUES, ONTOGENY, AND ADAPTIVE SIGNIFICANCE

INTRODUCTION

The distributions of all animals are in some way spatially and temporally restricted. Much of population and community ecology, historical and contemporary, has been concerned with explaining animal distributional patterns. Commonly, patterns are sought in predation pressure, competitors, food abundance, or climatic factors to explain distributions of species, populations, and individuals. In the present study, I was interested in the role of habitat choice as a proximate mechanism influencing distributions of frog larvae. Since Lack (1933) first emphasized the probable importance of behavioral preferences as mechanisms promoting habitat segregation in birds, habitat selection has received little rigorous examination outside theory.

The study of habitat selection, with few exceptions, has been approached in one of three ways. The "ecological" approach (or habitat correlation approach) is representative of most studies. A common objective of these investigations is to describe a species-typical habitat or to demonstrate and quantify habitat partitioning among potentially competing species (for examples of this approach see MacArthur 1958; MacArthur et al. 1962; Rand 1964; Schoener and Gorman 1968; Pianka 1969; M'Closkey 1976; Rose 1976; Dueser and Shugart 1978; Chappell 1978; Price 1978; Strijbosch 1979; Fenchel and Kolding 1979). Most often, certain environmental variables are measured (e.g.

vegetation composition and structure, climatic factors, soil characteristics) and statistically related to species abundance. Such descriptive studies may implicate certain factors as important in the habitat selection process, but they cannot be used to determine which factors animals are actually responding to or to rule out the contributory influence of alternative factors.

The "evolutionary" approach considers the ultimate factors (Hildén 1965) responsible in limiting animal distributions. Habitat selection behavior is viewed in a theoretical context as an outcome of competition, predator-prey interactions, or of abiotic constraints on physiology and morphology. If habitats differ in some quality affecting fitness, theory predicts that an organism should show habitat selectivity. Habitat selection in this sense is commonly viewed as a strategy of resource utilization, of predator avoidance, or of lessening intra- or interspecific competitive interactions (see discussions by Levene 1953; MacArthur and Levins 1964; MacArthur and Pianka 1966; Levins 1968; Fretwell and Lucas 1969; Bryant 1973; Rosenzweig 1974; Wiens 1976). Theory further predicts that if genetic variants (within a population) become habitat-specific (Jones 1980), continued habitat selection could lead to population divergence (Maynard Smith 1966).

The "behavioral" approach is primarily experimental and examines choice responses of organisms in controlled conditions to proximal cues or habitat types during development or following a period of conditioning. These investigators most often attempt to define the proximate behavioral causes of animal habitat distributions and how preferences become manifest during ontogeny of the organism. Although a

few experimental field studies have been attempted (Wecker 1963; Meseth and Sexton 1963; Kiester et al. 1975; Douglass 1976), most work has been done in the laboratory (Harris 1952; Gibb 1957; Klopfer 1963; Sale 1968; 1969, 1971; Wiens 1970, 1972; McKenzie and Storm 1970; O'Hara 1974; Partridge 1974; Jaisson 1980). In many of these studies, cues ("habitats") chosen by the experimenter are highly artificial, with little more than superficial attention given to the organism's life history or natural habitat. Consequently, much of this work is useful in pointing out behavioral potentials in choice behavior but may be of little direct applicability to habitat selection behavior in nature.

Clearly, no single approach has been adequate in addressing complex questions of how and why a particular organism exhibits habitat selectivity. To gain a thorough understanding of habitat selection as a behavioral process and as a strategy under the influence of natural selection requires information on natural history, quantitative descriptions of habitat and habitat usage, and experimentation to determine what key proximate factors are utilized by an organism in assessing habitat suitability and how preferences are ontogenetically established. Interpretations of adaptive value are perhaps most difficult to achieve operationally (Wiens 1976) and, in strict terms, require some measure of fitness prospects in preferred and less preferred habitats. While this is not impossible to attain for some organisms (Whitham 1978, 1980), it is a major undertaking for most and usually necessitates long-term study. The complexity and difficulty of thoroughly dealing with each of these conditions has resulted in the lack of a truly unified approach to studying the process of habitat

selection. To begin with, empirical studies that employ descriptive and experimental techniques in asking and addressing questions about habitat selection are needed.

In this study I investigate the mechanism of habitat choice in three species of anuran tadpoles: Rana cascadae (the Cascades frog), Hyla regilla (the Pacific tree frog), and Bufo boreas (the Western toad). These species overlap in distribution in the Cascade Mts. and were chosen to provide a comparative basis for analyzing habitat selection behaviors.

Anuran larvae are ideal subjects for the investigation of habitat selection behavior. Because tadpoles are aquatic, distributions are sharply confined and observational data are relatively easy to obtain in the field. The larval period is usually limited to a single season (2-4 months; these larvae do not overwinter) so a given population can be followed throughout larval development. Ovulation can be induced artificially in females in the laboratory and embryos and larvae can be reared easily. Experimental manipulations of tadpoles in respect to habitat type can readily be accomplished in the field and in the laboratory. Methods have been developed to test preference behaviors in tadpoles (Wiens 1970, 1972; O'Hara 1974), and tadpoles are known to be responsive to various physical and biological environmental factors (e.g. see Wassersug 1973).

Preliminary study of Cascade lakes and ponds suggested that these tadpoles were selective in where they spent their time relative to available habitat types. The major objectives of the study were to 1) define the proximate cues used by tadpoles in selecting a habitat; 2)

describe the ontogenetic basis of cue preference; and 3) delineate the possible selective constraints acting on the habitat selection process. The approach was an empirical one. Tadpoles were studied in their natural environment to discern patterns of association between species densities and measured environmental variables. This information was then used to formulate and test hypotheses in the laboratory regarding the ontogeny of cue preference.

## THE SPECIES

Rana cascadae

The Cascades frog inhabits montane marshes, streams, ponds, and lakes above about 1000 m altitude in the Cascade Mts. of northern California, Oregon and Washington and in the Olympic Mts. of Washington. Adults are closely restricted to relatively permanent bodies of water.

Breeding and egg laying activities ensue immediately following ice and snow melt from lake or pond margins and rarely last more than a few days. Sites selected for breeding are shallow, gently sloping margins of the shore or overflow areas, over soft substrates, and typically in areas protected from severe wave action. Adults utilize the same sites for breeding each spring (personal observations over 5 yr). Females oviposit single globular egg masses comprising 300-500 eggs, often next to or on top of conspecific egg masses. Larvae are primarily bottom dwellers and feed on detritus, suspended particulates, aufwuchs (periphyton), and (opportunistically) on dead animal tissue.

Hyla regilla

The Pacific tree frog ranges from British Columbia to lower California and extends into Idaho and Nevada. Adults are usually found in the vicinity of water from sea level to over 3300 m altitude, from arid open to heavily forested areas.

Breeding in the high Cascades occurs shortly after the ice melts from water surfaces and shore margins and may continue sporadically for



2-3 weeks. Females lay several egg clusters of 10-70 eggs each and frequently attach them to vegetation, sticks, or other objects in shallow water. Tadpoles are bottom dwellers and feed on detritus, suspended particulates, aufwuchs, and dead animal tissue.

### Bufo boreas

The western toad is distributed from southern Alaska to northern Baja California, from the Rocky Mts. to the Pacific Coast. It is found throughout most of Oregon, with the exception of the Willamette Valley. The species occurs in a wide variety of habitats including desert springs and streams, grasslands, and montane meadows and lakes. Adults are wide ranging and may be found at considerable distances from water.

In the high Cascades, breeding activity ensues shortly after the ice recedes and melts from shore areas in late May to mid-June and lasts 2 weeks or less. Marshy areas or the shallow margins of lakes and ponds are preferred oviposition sites (personal observations). Females lay an average of 12,000 eggs (Samollow 1980) in strings in shallow water. Eggs are often intertwined around twigs, logs, or vegetation and clutches from several females are usually deposited in one small area.

Larvae often reach very high densities (compared with other species) and are noted for forming large and elongate polarized schools and compact aggregations along shoreline. Tadpoles are primarily particulate suspension filterers but will feed on bottom detritus, aufwuchs, and dead animal tissue.

## FIELD STUDIES

### Study Sites

I surveyed potential field study locations during the summer of 1975. The ideal study site was considered to be one possessing the following attributes: 1) breeding populations of all three species; 2) easy access; 3) minimal human disturbance; 4) a permanent body of water; 5) an aquatic environment offering several distinct habitat types; and 6) a shore terrace and water conditions amenable to sampling and observation.

One site, Little Three Creek Lake, satisfied all prerequisites. It is located in Deschutes County, Oregon (T17S, R9E, Section 15) at an altitude of 2,045 m just east of the Cascade Crest. The lake is approximately 20 ha in size (spring high water) and is fed by precipitation (primarily snow) and springs and is drained by a small creek at its northern end (Figure 1).

Little Three Creek Lake (LTC) is snow- and ice-bound from late October or November to late May or June. Emergent clumps of sedges, Carex, comprised the dominant vegetation type along gently sloping moist areas of the shore margin and flooded shallows in early summer (Figure 1). The only other aquatic macro-vegetation of significance was the grass-like quillwort, Isoetes. It was prevalent in soft, gently sloping substrates of the shore terraces and covered the bottom in carpet-like fashion becoming thicker and taller as the summer progressed. Shore terrace substrates ranged in particle size from loose flocculent mud (fine substrates) through sand, gravel, and rock (coarse substrates).

## Little Three Creek Lake

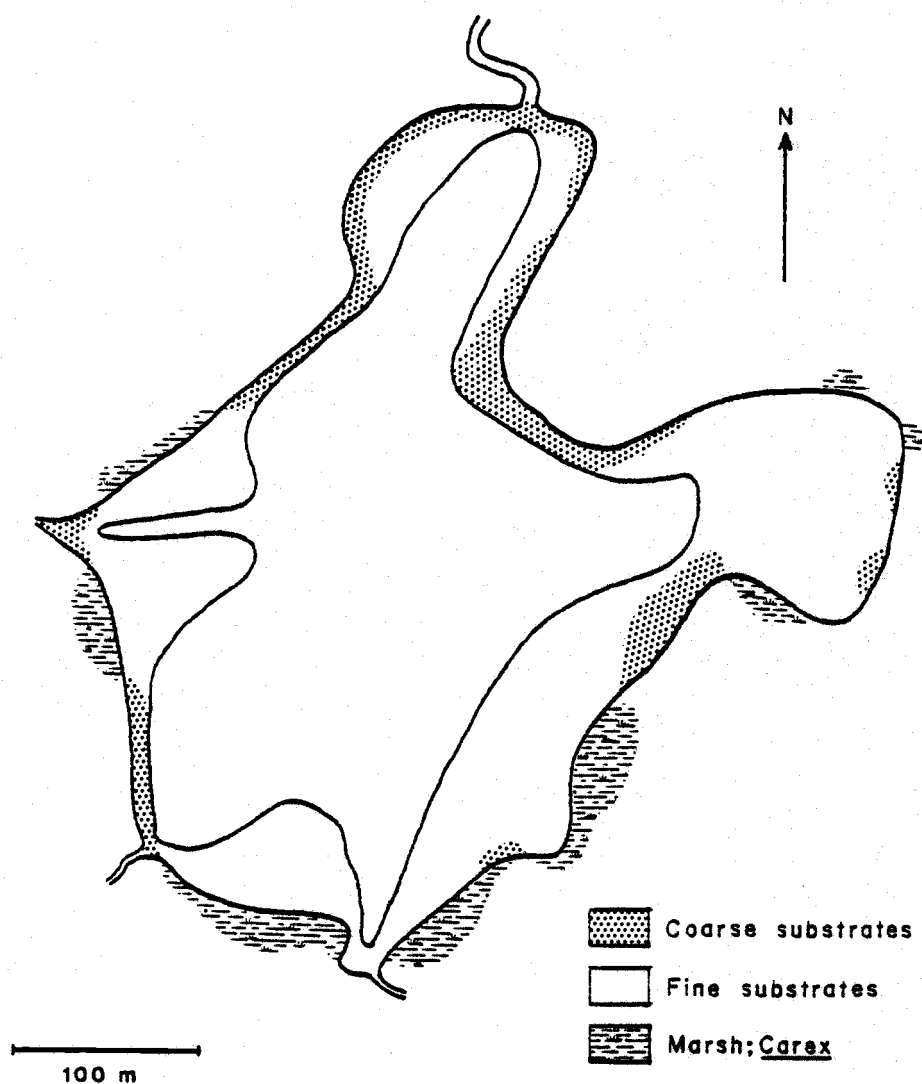


Figure 1. Major vegetational and substrate features at Little Three Creek Lake at spring high water. Inner margin denotes water depth at approximately 1 m.

Snags of downed logs and stumps were common in places along lake shores and shallows.

I concentrated most of my field efforts at LTC. Secondary sites of frequent visitation where no intensive sampling was initiated included Todd Lake, Deschutes County (T18S, R9E, Section 8), at an altitude of 1,875 m, and Green Lakes, Deschutes County (T17S, R8E), at an altitude of 1,985 m. Approximately 25 other localities were surveyed for tadpole habitat distributional patterns during the course of the study.

## Habitat Associations

The main objective of the field work was to quantify information relating species distributions with environmental variables. This would provide a basis for formulating and testing hypotheses about single factor behavioral responses of tadpoles in the laboratory.

Quantitative field data are available for 1976 only. In 1977, a drought severely affected the LTC frog populations and breeding was depressed that year in all species. All Rana tadpoles disappeared from the lake (probably due to mortality; several dead tadpoles were found) by mid-July, Hyla tadpoles were few in number and decreased rapidly through the summer, and mortality in Bufo tadpoles was much higher than in previous years. The collection of field data was hindered because of drastic drops in water level on a weekly basis and the resulting uniformity of habitat around the entire lake perimeter by July. Returns to LTC in 1978, 1979, and 1980 have indicated that the Rana population became extinct in that area following the drought and has not yet returned. Breeding by Hyla has been much reduced over initial observations made in 1975 and 1976.

## Methods

Sampling Techniques. One sampling technique was instituted in June. The shore was used as a transect line and temporary plots (0.75 m on a side) were situated around the lake perimeter at three distances from the shore (0, 2, and 6 m). A change in sampling procedures was prompted by the widespread destruction of plots (approximately 60 to 70%) in the

interim between two of my visits in early July. Methods and analysis techniques were changed in order to utilize the information already collected and to prevent further human interference with plots.

This new procedure was based on the observation that data collected along certain continuous sections of shoreline were relatively uniform in measured habitat variables. The largest and most consistent variations in species densities and habitat variables were between these major areas.

I subjectively defined ten perimeter sampling sites around the lake perimeter at LTC (Figure 2). Within each area for a given distance from shore, substrate type, water depth profile, and vegetation type and abundance were relatively uniform. Each site was quantitatively assessed for these attributes four times during the summer.

The unit area within which individual observations were taken was  $0.56 \text{ m}^2$ . Within perimeter sites, specific localities for observations at each sampling bout were randomly determined. A random numbers table was used to dictate the number of paces to take along the shoreline. Opposite that point, data on time of day, temperature, species density, and dissolved oxygen were taken at each of three shore distances. The lake was visited nearly every week during the summer for a 2-5 day period each visit. This sampling method may have been less resolute in the detection of patterns at the level of microhabitats than sampling from small semipermanent plots would provide, but was probably better at magnifying the major trends in tadpole habitat associations.

Variables and Measurement. Each measured variable was known or suspected to influence distributions of tadpoles and each was amenable

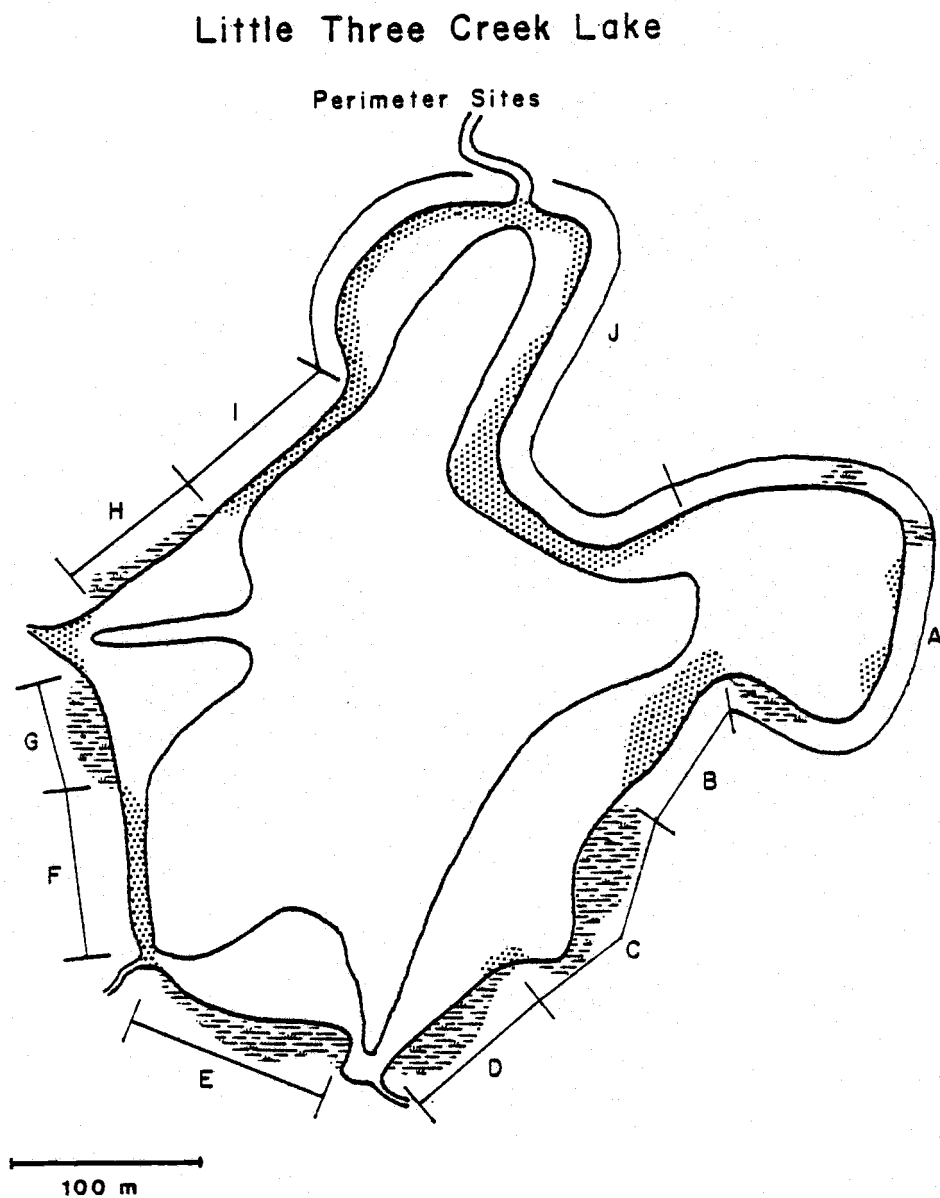


Figure 2. Positions of the 10 perimeter sampling sites, A-J, at Little Three Creek Lake.

to measurement in the field (Table 1). Estimates of water depth, substrate, and vegetation (for each shore distance of each perimeter site) were taken once early (period I) and once late (period II) in each of two summer periods to account for seasonal changes in water level drop and vegetation growth. Summer period I extended from 25 June to the end of July, period II from August to 15 September. Data were averaged for all variables over each summer period and independently analyzed.

A 1-m rule was used to measure water depth. Average depths for each shore distance (henceforth abbreviated ShD) were determined by taking readings at every 4-5 m along the shore at ShD's of 0.3, 3.5, and 8.0 m (for sites F and I readings were made at 0.2 and 1 m) within each perimeter site. Oxygen tension and temperature were measured with a YSI Model 51 probe. Percent coverage of each substrate type was estimated for each ShD of each perimeter site. Readings were taken twice for each summer period (7 and 27 July for period I and 9 August and 11 September for period II). Surface area coverage of rare substrate types was directly measured and dominant substrate types were estimated visually at each perimeter site.

Precise assessments of vegetation percent coverage were not possible due to large variations in vegetation density and patchiness and time constraints. Vegetation classes were assigned a rank (0 to 5) to reflect overall density and abundance of that class relative to conditions at all other sites. Species density estimates at each perimeter site ShD were assigned to a density class (Table 1) at the time of observations. Bufo were far more numerous than Rana or Hyla,



Table 1. Habitat variables, categories, and units of measure recorded at perimeter sites at LTC.

<u>Variable</u>	<u>Categories/Units of Measure</u>
Summer Period	1. June-July 2. August-September
Perimeter Site	Ten sites designated A through J
Time of Day	1. 0600-0900 2. 0900-1200 3. 1200-1500 4. 1500-1800
Distance from Shore	1. 0 to 1 m 2. 1 to 5 m 3. 5 to 10 m
Water Depth	Centimeters
Water Temperature	Degrees C
Dissolved Oxygen	mgm/liter
Substrate	Estimated % Coverage 1. Mud (fine particulate) 2. Sand 3. Gravel (<10 mm ave. diameter) 4. Rock (>10 mm ave. diameter)
Vegetation	Each class assigned a rank of 0 to 5. 0 = not present; 5 = high relative % coverage. 1. Open (no vegetation) 2. <u>Isoetes</u> 3. <u>Carex</u> 4. Log (logs or wood debris)
Tadpole Density	<u>Rana</u> or <u>Hyla</u> (number/.56 m <sup>2</sup> ) 1. 0 2. 1 to 5 3. 6 to 10 4. 11 to 20 5. 21 to 40 6. 41 or greater  <u>Bufo</u> (number/.56 m <sup>2</sup> ) 1. 0 2. 1 to 5 3. 6 to 15 4. 16 to 40 5. 41 to 80 6. 81 to 150 7. 151 to 250 8. 251 or greater

and was given a different classification scheme. For purposes of analyses, the median of each class range was multiplied by the number of observations in that class and these values were summed over all density classes and divided by the number of observations to obtain the mean density/.56 m<sup>2</sup>.

Statistical Analyses. Data were analyzed on the OSU computer using the Statistical Interactive Programming System. Data from each summer period in 1976 were analyzed independently. Each of the two data sets consisted of four blocks corresponding to the time of day observations were taken. Numbers for each variable were represented mean values for a shore distance area at each perimeter site. Each block was made up of 25 variables (columns). These were densities for three species, 10 site localities (designated as indicator variables), and 12 environmental variables. Each variable in a block had 28 values (rows) corresponding to the mean values at each ShD of each site (for two of 10 sites data were taken at only two ShD's due to a sharply sloping shore terrace).

Field data were subjected to two statistical analyses. Correlation analysis was used to describe the nature of the association between patterns of tadpole densities and given environmental variables. It was also used in pointing out associations among the independent variables and in selecting the final independent variables to be included in a regression model. Product-moment correlation coefficients were calculated for all pairs of dependent (species densities) and independent variables for each summer period.

Regression analyses enabled me to assess the role of variables selectively or collectively in explaining the observed variation in the

dependent variables. The primary purpose in using regression techniques in this study was not to present a numerically precise model to account for tadpole distributions, but rather to delineate between potentially significant and nonsignificant environmental variables relating to habitat selection behavior.

Multiple linear regression by least squares approximation (Neter and Wasserman 1974) was used to describe the relationship between dependent variables (tadpole densities) and measured independent variables. In order to utilize linear functions in describing the data, the residuals (error terms) of selected models should be independent, normally distributed, with a mean of zero, and a constant variance. By examining residuals for these properties, primarily through graphical techniques (Neter and Wasserman 1974), it was determined that the assumptions of linearity were valid in these analyses. Rana was not included in regression analyses because this species was present in only two of 10 perimeter sites throughout the summer.

#### General Patterns

The following summarizes some general trends in daily and seasonal variation of measured environmental parameters. These trends largely dictated the procedures for data collection and analysis.

Oxygen content and water temperature are two variables that undergo large diel fluctuations along the shore terrace at LTC. Fluctuations in oxygen largely reflect the life processes of the plant biota (e.g. see Ruttner 1963). At low light levels, respiratory activities remove oxygen from the water, resulting in gradual depletions during nighttime

hours with lowest deficits occurring in pre-dawn hours. As light intensity and temperatures increase, photosynthetic activities rapidly replenish dissolved oxygen, sometimes approaching super-saturation in extreme shallows. Tadpoles themselves are capable of significantly altering oxygen profiles. Levels in the midst of Bufo aggregations were commonly much lower than ambient. The greatest daily fluctuations in magnitude at LTC occur in the shallow shore margins. These patterns are quite clear as depicted by representative field data in Figure 3.

Water temperature undergoes a more definable cyclic pattern as it is less influenced by biological activities. The primary factors affecting temperature are solar input, the air-water temperature gradient, and wind and wave action. Ambient air temperatures commonly approached the 0-10°C range at night and sometimes exceeded 30°C during midday. The shallower the water, the greater the variability in temperature throughout a diel cycle. Deeper waters take longer to warm and cool and the magnitude of fluctuations is increasingly damped with greater depth. Tadpoles affected water temperatures to a limited extent. In dense Bufo aggregations temperatures reached as high as 2-3°C over the surrounding water. A representative temperature profile (Figure 4) serves to illustrate patterns of temperature change throughout a day. These diel patterns in temperature and oxygen fluctuation emphasize the importance of daily time periods in the collection of data.

There were also seasonal changes during a summer at LTC. Data were analyzed according to summer period in order to account for seasonal effects. Although no major trends were notable in oxygen profiles between periods, temperatures undergo a warming trend from June into

## PERIMETER SITE D

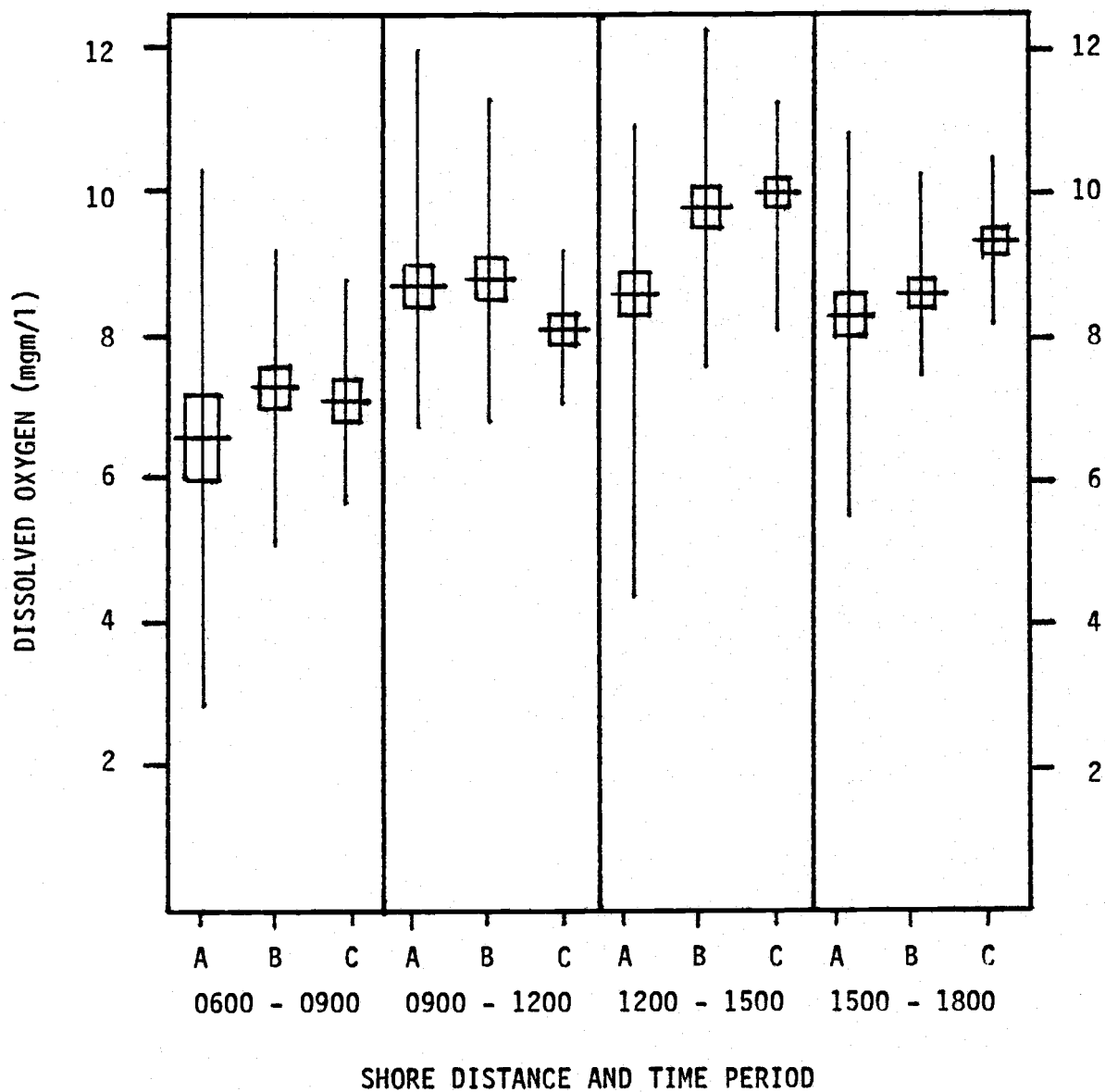


Figure 3. Representative graph of means, 95% confidence intervals, and ranges of dissolved oxygen at perimeter site D. Readings are categorized in respect to time of day and shore distance (A = 0-1 m; B = 1-5 m; C = 5-10 m).

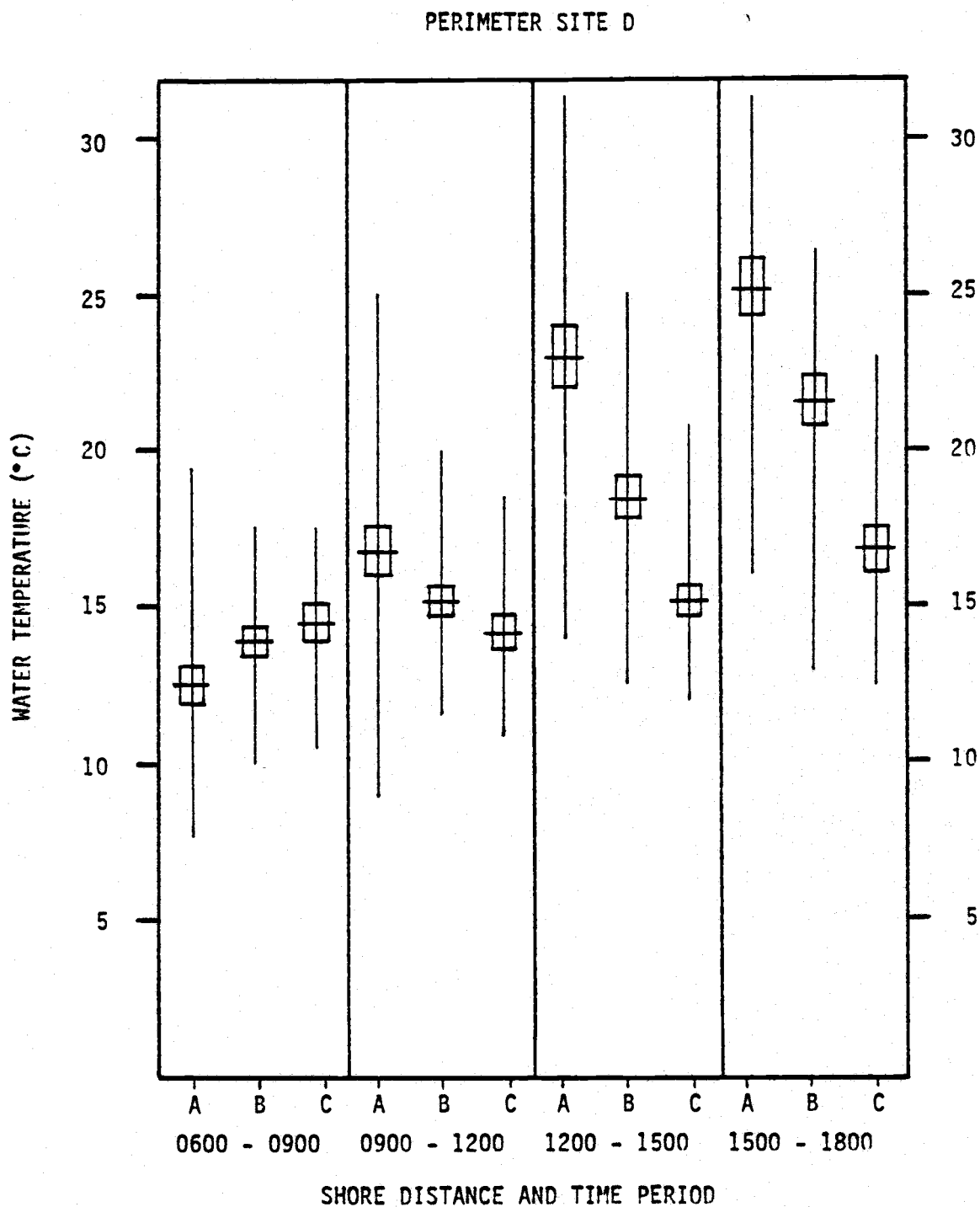


Figure 4. Representative graph of means, 95% confidence intervals, and ranges of water temperature at perimeter site D. Readings are categorized in respect to time of day and shore distance (A = 0-1 m; B = 1-5 m; C = 5-10 m).

August, when the trend begins to reverse. Aquatic vegetative characteristics change, partly due to gradual drops in water level and partly to patterns in growth and reproduction. Water level drops effect a gradual elimination of the land-water interface species (i.e. Carex) from the aquatic environment. Through most of the latter summer period Carex was no longer a conspicuous element at most perimeter sites. Log and wood debris, localized at the water's edge in early summer, also diminished in representation as the water receded. The most significant and rapid changes in macro-vegetation growth occurred in the first summer period. Carex, which appears brown and dead immediately after spring thaw, grows to 10-25 cm in height by mid-summer. Isoetes ranges from 1-8 cm in height.

Changing water level also affects the physical configuration of the shoreline and substrate representations at each perimeter site. This change was greatest in July and early August. Sand, gravel, and rock substrates were limited to within approximately 5 m of the shoreline at spring high water. Consequently, these substrates decrease in frequency throughout the summer and shore terrace substrates become increasingly uniform (mud) in composition. Substrate composition was also influenced by tadpole activity. Fecal material came to form a major element where tadpoles were most concentrated.

## Results

The raw data sets that served as input for computer analysis are given in Appendix I. The objective of this analysis was to select those variables most consistently related to patterns of species densities.

Correlation Analysis. Product-moment correlation coefficients for all relevant variables for each summer period are given in Appendix II. A combined summary table is presented here (Table 2) of all significant (at  $P < 0.05$ ) correlation coefficients. Despite seasonal changes in climate and habitat composition and structure, correlations among variables were highly similar between summer periods. An examination of significant correlation coefficients shows widespread intercorrelations among variables. Every variable (excluding site localities and oxygen in period I) is significantly correlated with six or more other variables. These relationships are perhaps not surprising when one considers the pattern of variation among the variables. Although there is considerable variability between perimeter site localities, the most notable trends are with increasing distance from shore. Because every variable changes the shore distance (and because ShD was a major criterion for measurement), intercorrelations will be high whether or not functional relationships exist between variable pairs. Also, certain variable are affected by others simply by their physical presence. The presence of any one substrate type automatically determines to some degree the occurrence of the other three. "Open" habitats were found where vegetation was lacking and closely corresponded to the presence of the firmer substrates.

Bufo. Variables correlated with Bufo densities in both periods were temperature, depth, rock, Isoetes, and Carex. Temperature was the variable most strongly associated with density ( $r = .72$  and  $.69$ ). Shore distance and log variables were correlated with Bufo in period I but not



Table 2. Matrix of significant ( $P < 0.05$ ) correlation coefficients among sampled variables at LTC for both summer periods combined.

<u>BUFO</u>	<u>HYLA</u>	<u>RANA</u>	<u>SHD</u>	<u>TEMP</u>	<u>O<sub>2</sub></u>	<u>DEPTH</u>	<u>MUD</u>	<u>SAND</u>	<u>GRAV</u>	<u>ROCK</u>	<u>OPEN</u>	<u>ISOETES</u>	<u>CAREX</u>	<u>LOG</u>	
1.000	.682	.212	-.199	.696	.174	-.348	.162			-.216	-.172	.313	.400	.255	<u>BUFO</u>
	1.000	.368	-.482	.538		-.552							.634	.353	<u>HYLA</u>
		1.000	-.242	.206		-.268							.415	.500	<u>RANA</u>
			1.000	-.280	.191	.644	.475	-.489	-.368	-.267	-.600	.500	-.554	-.413	<u>SHD</u>
				1.000	.282	-.369							.254	.159	<u>TEMP</u>
					1.000	.213									<u>OXYGEN</u>
						1.000	.437	-.473	-.336	-.229	-.361		-.617	-.492	<u>DEPTH</u>
							1.000	-.836	-.898	-.590	-.822	.722	-.213	-.320	<u>MUD</u>
								1.000	.818	.147	.736	-.590	.396	.478	<u>SAND</u>
									1.000	.239	.640	-.600		.352	<u>GRAVEL</u>
										1.000	.558	-.494			<u>ROCK</u>
											1.000	-.790	.363	.364	<u>OPEN</u>
												1.000		-.278	<u>ISOETES</u>
													1.000	.556	<u>CAREX</u>
														1.000	<u>LOG</u>

in period II. This probably reflects the greater dispersion of Bufo (from sites of oviposition) around the lake perimeter and across ShD's in period II. The variable open, indicating vegetation absence, was negatively correlated with Bufo in period II but not in period I. This result suggests a decreasing presence of open patches through the summer due to the combined effect of vegetation growth and receding water level. The areas that remained open by period II were over the harder substrates which tended to be avoided by tadpoles.

Hyla. Variables positively correlated with Hyla in both summer periods were Carex, temperature, and log (wood debris). Those negatively correlated were ShD and depth. Tadpoles were never recorded beyond 36 cm depth at LTC. Consistency in magnitude and direction of correlation coefficients between periods can be attributed to low dispersal of this species between sites combined with a relatively uniform change in variable representation across sites through the summer. The fact that Hyla frequents the gently sloping shallows where Carex is more abundant is reflected in high correlation coefficients for this variable ( $r = .83$  and  $.72$ ), and for temperature and depth.

Rana. Rana was present in only two of 10 perimeter sites (A and D) throughout the summer. Consequently, variable correlations (which are based on differences between sites) emphasize the qualities these two sites have in common and that tend to differ from the other sites. This same interpretation can be applied to the significance of the variable Carex. Significant associations with ShD, temperature, and depth

reflect distributions biased toward the shallows. Rana were never observed in the most distant ShD category or beyond 45 cm depth.

The lack of correlation of substrate type with species densities (Table 2) is misleading. Hyla and Rana tadpoles were actually found exclusively over mud substrates. Bufo tadpoles were recorded only rarely over substrates other than mud. But because data were averaged in respect to perimeter site, and because most perimeter sites contained patches of all substrate types, this clear relationship with substrate was not distinguished in the analysis.

Multiple Regression Analysis. When independent variables are intercorrelated, as in the present case, the selection of a best regression model is complicated, and many variable combinations may provide equally suitable models in describing the data. For a discussion of these considerations, as applied to the present data set, see Appendix III.

Bufo. In early summer, temperature was the only variable to provide significant reduction in variance of tadpole density regardless of which variables were included in the mode (Table 3). It was the best single predictor of tadpole densities. Temperature alone explained over half of the variation in Bufo density (coefficient of multiple determination  $R^2 = .52$ ).

Variable selection following inclusion of temperature was largely dependent on whether substrates were collectively included or not. With substrates and temperature, the best model was achieved by the addition

Table 3. Summary of significant multiple regression models of Bufo densities on environmental variables in early and late summer periods.

Regression Equation		MSE	R <sup>2</sup>	F**
<u>Early Summer</u>				
<u>Bufo</u> = - 56.1	+ 3.42 (Temperature) - 0.18 (Depth) +27.95 (Mud) +20.96 (Gravel) -13.57 (Rock)	132.55	.64	36.91
<u>Bufo</u> = - 38.9	+ 3.33 (Temperature) - 1.93 (Shore Distance) - 4.04 (Open) + 3.61 (Isoetes) + 2.37 (Log)	115.13	.68	45.70
<u>Bufo</u> = - 54.8	+ 3.32 (Temperature) + 3.96 (Isoetes) + 3.10 (Carex)	124.31	.65	67.27
<u>Bufo</u> = - 37.1	+ 3.38 (Temperature) - 4.12 (Open) + 3.99 (Carex)	123.64	.65	67.83
<u>Late Summer</u>				
<u>Bufo</u> = -109.7	+ 3.22 (Temperature) - 0.20 (Depth) +82.24 (Mud) -39.36 (Sand) +70.35 (Gravel)	76.02	.69	48.16
<u>Bufo</u> = - 63.1	+ 3.29 (Temperature) + 5.38 (Isoetes) + 2.95 (Log)	87.35	.64	64.60
<u>Bufo</u> = - 30.6	+ 3.23 (Temperture) - 0.12 (Depth) - 7.42 (Open)	89.78	.63	61.87
<u>Bufo</u> = - 58.0	+ 3.18 (Temperature) + 4.87 (Isoetes) + 3.03 (Carex)	89.38	.63	62.31

\*\* All F-values are significant at P < .001.

of depth (Table 3). In the absence of substrates, several models of near equal fit were possible. Vegetation variables (open, Isoetes, Carex, and log) can be considered as counterparts in the model for substrates, depth, and ShD by virtue of their association with the latter. Oxygen was never a significant regression variable and the variable log rarely contributed to a model.

Selected models for late summer (Table 3) bear close similarities with models in early summer except that some variable combinations differed. Temperature again was the dominant variable, and with substrates in the model the addition of depth again provided the best fit. In the absence of substrates, several alternative variable combinations gave similar fits.

Hyla. Carex was the best single predictor of Hyla densities (Table 4). With Carex alone, most of the variation in Hyla density was accounted for (period I,  $R^2 = .69$ ; period II,  $R^2 = .51$ ). In both periods, temperature was the next best predictor. In period I, there were two models providing equally good fits that were appropriate in describing the data (Table 4). In one model, depth and open variables were combined with temperature and Carex, in the second, substrates were matched with the two primary variables. No combination of other independent variables satisfactorily explained Hyla density in this summer period.

In period II, Carex and temperature were again the primary predictors but substrates also significantly explained variance in Hyla (Table 4).

Table 4. Summary of significant multiple regression models of Hyla densities on environmental variables in early and late summer periods.

Regression Equation		MSE	R <sup>2</sup>	F**
<u>Early Summer</u>				
<u>Hyla</u> = - 4.5	+ 0.24 (Temperature) + 1.34 (Carex) + 0.01 (Depth) - 0.26 (Open)	1.16	.82	120.69
<u>Hyla</u> = - 5.9	+ 0.23 (Temperature) + 1.21 (Carex) + 2.19 (Mud) - 3.16 (Sand) + 4.11 (Gravel)	1.23	.81	90.08
<u>Late Summer</u>				
<u>Hyla</u> = -8.3	+ 0.19 (Temperature) + 2.96 (Carex) - 0.28 (Shore Distance) + 4.05 (Mud) -34.52 (Sand) +30.04 (Gravel) + 0.74 (Isoetes) + 0.56 (Log)	2.31	.76	40.80
<u>Hyla</u> = -11.1	+ 0.20 (Temperature) + 3.07 (Carex) - 0.03 (Depth) +10.35 (Mud) -31.95 (Sand) +33.59 (Gravel)	2.53	.73	47.79
<u>Hyla</u> = -0.9	+ 0.23 (Temperature) + 1.95 (Carex) - 0.38 (Shore Distance) - 1.38 (Open)	3.05	.67	54.50

\*\* All F-values are significant at P < .001.

## Estimates of Dispersal for Rana and Hyla Tadpoles

Relevant to considerations of habitat selection behavior are patterns of dispersal between habitats. Field observations of tadpoles suggested that the three species exhibit varying degrees of dispersal. Bufo tadpoles were high dispersers. They were recorded in all habitat types available at LTC and schooling tadpoles were active in deeper water than the other species. Rana and Hyla tadpoles appeared to be far more sedentary and remained in the vicinity of oviposition sites.

### Methods

To estimate dispersal rates between habitats and between perimeter sites, a staining technique was used to mark tadpoles (Herreid and Kinney 1966; Guttman and Creasey 1973). Tadpoles were collected, immersed in a solution of neutral red dye, and then released at particular localities in the field. Stained tadpoles of Rana and Hyla remained perceptibly red for 3-6 days in the field, during which time their locations could be monitored. The staining technique was ineffective in producing an observable difference in the black bodies of Bufo tadpoles.

Estimated mortality of tadpoles directly attributable to the staining technique was 0 to 25%. This would occur within the first 2-4 h after release. Other individuals, although initially sluggish, recovered as if unaffected. All marking experiments took place in July and August. Most tadpoles marked were approximately midway through larval development (stages 28-38; Gosner 1960).

Groups of dyed tadpoles were either released where they were captured or were released elsewhere along the shore of the same lake. When translocated to another site, tadpoles were either released in a habitat similar to the one in which they were collected (preferred habitat) or one different from the collection site (nonpreferred habitat). Studies were carried out at LTC, Todd Lake, and Green Lake. The latter studies extended over 2-day periods only.

Following release of tadpoles, the shore terrace for at least 30 m on either side of the release point was censused visually twice daily (morning and afternoon) until no more marked animals were observed or until I vacated the site. Information recorded included the number of marked tadpoles observed and the maximum distance along the shoreline in meters that any tadpole was found on either side (left or right) of the release point.

## Results

Dispersal limits for tadpoles released at the point of capture (Table 5) clearly show an absence of movement from the vicinity of the "home" area. The maximum range in linear distance recorded for both species was 7 m and mean distances dispersed ranged from 3.5 to 5.4 m. Distance ranges were constant between species and between sampling periods within each series. Some dispersal limits were correlated with discontinuities in habitat types (e.g. deep water, cold temperatures, coarse substrates). In these cases, such discontinuities may have represented barriers to dispersal.



Table 5. Summary of dispersal estimates of Rana and Hyla tadpoles that were marked, released at the capture site, and visually censused twice a day.

Species	Locality	No. Marked and Released	% Marked Tadpoles Observed and Maximum Dist. Recorded (m) Either Side of Release Point								Mean Dist. (m) Dispersed	Max. Dist. (m) Dispersed
			Sampling Period									
			1	2	3	4	5	6	7	8		
<u>Rana</u>	LTC Site A	40	85 1,1	65 2,3	68 2,3	50 2,5	48 2,4	38 2,5	30 2,4		5.4	7
	LTC Site D	40	70 2,1	78 2,3	60 3,2	48 2,2	35 3,1	43 2,2	23 3,2		4.3	5
	LTC Site D	40	90 1,1	78 2,2	58 2,1	68 3,2	50 2,2	40 2,1	28 2,2	18 1,2	3.5	5
	Todd L.	50	78 1,2	54 2,3	58 3,3	44 2,4					5.0	6
	Green L.	50	68 1,1	46 2,2	42 4,2						4.0	6
<u>Hyla</u>	LTC Site C	70	76 2,2	56 3,2	43 3,3	43 4,2	26 3,3	17 3,1			5.2	6
	LTC Site G	70	80 1,2	64 2,3	44 4,3	37 3,1	36 2,2	27 3,0	14 2,1		4.1	7
	Todd L.	75	83 2,1	71 2,4	47 2,2	41 1,3					4.2	6

The downward trend in numbers with time can be largely attributed to differential mark loss (my own lab experiments with mark longevity show that some individuals lose the mark sooner than others), partially to the failure to detect animals that were present, and perhaps loss to predators (not directly observed). Loss due to dispersal from the sampled area is unlikely. Not only were no marked tadpoles observed outside the limits indicated, but no marked tadpoles were observed at any other localities around the lake perimeters during the experiments.

Tadpoles were also dyed and translocated to different sites within the same lake (Table 6). When tadpoles were released in "preferred" habitats that were similar to the capture site (designated mud), dispersal distances were no different from tadpoles released at the capture site (Table 5). Tadpoles translocated and released in "nonpreferred" habitats (designated sand, gravel, or rock) exhibited greater mean and maximum distances dispersed than those released at capture sites. Furthermore, when the disappearance rates of marked tadpoles per sampling period are compared, tadpoles translocated to nonpreferred habitats disappeared at a faster rate ( $\bar{x} = 35\%$ ) than those released at the capture site ( $\bar{x} = 21\%$ ) (significant at  $P < 0.05$ ; test for equality of two percentages, Sokal and Rohlf 1969). The source of higher disappearance rates could be due to a greater susceptibility of tadpoles to fish (Salvelinus) predation in these sites. Water was generally deeper and the shoreline had a steeper slope in nonpreferred habitats such that the incidence of fish was higher.

Table 6. Summary of dispersal estimates of Rana and Hyla tadpoles that were marked, translocated to either a preferred (mud) or non-preferred (sand, gravel, rock) habitat type and released, and visually censused twice a day.

Species	Locality	No. Marked and Released	% Marked Tadpoles Observed and Maximum Dist. Recorded (m) Either Side of Release Point							Mean Dist. (m) Dispersed	Max. Dist. (m) Dispersed
			Sampling Period								
			1	2	3	4	5	6	7		
<u>Rana</u>	LTC Site A to C (Mud)	30	80 2,2	63 5,3	53 4,6	37 2,3	27 0,5	10 0,4	0	6.0	10
	LTC Site D to I (Rock)	30	73 1,2	60 4,5	17 6,3	0	0			7.0	9
	Todd L. (Mud)	50	86 1,1	60 2,3	48 2,2	42 1,2				3.5	5
	Todd L. (Sand)	50	64 4,3	30 7,2	16 9,0	0				8.3	9
<u>Hyla</u>	LTC Site C to G (Mud)	70	83 1,3	67 2,5	47 2,3	34 3,5	23 3,2	14 3,1		5.5	8
	LTC Site C to I (Rock)	70	63 4,5	47 5,3	27 8,6	20 5,9	9 0,11	3 0,6		10.3	14
	LTC Site E to J (Gravel)	70	56 3,6	36 5,8	31 2,8	19 0,9	13 0,8	13 0,8		9.5	13
	Todd L. (Gravel)	75	64 5,3	56 7,5	40 8,7	36 7,7				12.2	15

Some dispersal patterns of translocated tadpoles were also associated with discontinuities in habitat. In one case (Todd Lake Rana released in a preferred habitat type), the patch of habitat extended for approximately 7 m along the shoreline and was bordered on either side by a sand or sand-gravel substrate. These habitats seemed to represent barriers to dispersal outside the preferred area. When tadpoles were transferred to a similar habitat of larger area (LTC Rana translocated from site A to C) dispersal was greater. Some Hyla tadpoles released over a gravel substrate in site J at LTC dispersed to a suitable habitat and seemed to stay there. This is illustrated by the dispersal distance readings to the right of the release point. This area was approximately 7 m from the point of release and was reached by some larvae within 24 h.

These experiments corroborated general field observations. Dispersal of Rana and Hyla tadpoles from patchily distributed oviposition sites was low, tadpoles tended to disperse more from nonpreferred habitats than from preferred habitats, disappearance was greater in nonpreferred habitats, and patches of nonpreferred habitat seemed to represent barriers to dispersal.

## Benthic Food Analysis

Substrate type was one variable strongly associated with tadpole distributions. All species were recorded predominantly over soft, muddy substrates at all pond and lake localities visited. It was hypothesized that substrate could be an important cue if it is related to food abundance. These larvae are primarily benthic suspension feeders and grazers and do not feed directly on Carex and Isoetes. To estimate potential food available to tadpoles, I collected benthic samples from various substrate types at LTC and analyzed them for organic and chlorophyll content.

## Methods

Two procedures were used to estimate quantities of substrate bound food. One provided an estimate of organic matter present in the form of filterable volatile solids. The second measured levels of chlorophyll a to yield an estimate of algal standing crop. Both methods of analyses are standard limnological procedures (for example see Lind 1979).

Benthic samples were collected for analysis in August 1978 and July 1979 from LTC. Samples were collected from different perimeter sites such that the spectrum of available shallow water habitats would be represented. For each site, sampling locations were determined by selecting a number from a random numbers table and pacing from the edge of the perimeter site along the shoreline. Two or three samples were then taken within an area of about 25 x 25 cm at a depth of 10-15 cm within 1 m of the shore. A tin can (73 mm diameter) open at both ends

was used to isolate an area of the substratum for each sample. A suction device (mouth diameter of 4.5 mm) was used three times for each sample, providing an average volume of 93 ml. The substrate type was recorded and samples were labeled, sealed, chilled in the dark, and immediately transported to the lab where they were kept in the dark at 4°C until analysis.

For each sample, particulates were concentrated with a Millipore Filter on a 0.45  $\mu$  pore size glass fiber filter. One-half of each filtrate was then analyzed for volatile organics, the other half was frozen in the dark and later analyzed for chlorophyll a.

For organics, samples were dried, stored in a desiccator, and weighed (all weighing was made on the same Mettler H20T balance). Dried samples were ignited at 525-550°C in a muffle furnace and the ash-free weight determined.

To estimate chlorophyll a levels, samples were macerated and transferred to centrifuge tubes with at least 5 ml of 90% reagent grade acetone. These were allowed to steep for 24 h in the dark at 4°C and then centrifuged at 500 g for 10 min. Supernatants were decanted and volumes determined. Optical densities at 663 and 750 nanometers (the latter a correction for turbidity) were read on a Beckman Model 24 spectrophotometer and reread following acidification with one drop of 1 N HCl (for pheophytin a correction). Pheophytin is a physiologically inactive degradation product of chlorophyll and may be present in phytoplankton. It can be a source of error in chlorophyll estimates because its absorption peak overlaps with that of chlorophyll a. Acidification converts chlorophyll to pheophytin thus allowing one to

estimate levels of pheophytin in a sample. An optical density ratio of 1.70 (at 663 nm before acidification/663 nm after acidification) indicates a sample is free of pheophytin and probably contains a nondecaying algal population. Formulas used to calculate pigment concentrations are as follows:

$$\text{Chlorophyll } \underline{a} \text{ (ug/l)} = \frac{26.7 (663_b - 663_a) \times E}{V \times L}$$

$$\text{Pheophytin } \underline{a} \text{ (ug/l)} = \frac{26.7 (1.7 \times 663_a - 663_b) \times E}{V \times L}$$

Subscripts a and b denote optical density readings after and before acidification; E the volume (ml) of acetone used; V the volume (liters) of water filtered; and L the path length of light traveled through a sample (1 cm).

To make comparisons among these data, some assumptions regarding seasonal and yearly variation in variables were made. There was no way of knowing if levels of organics and chlorophyll differed between years. As values were to be used as relative indices rather than absolute quantities, an assumption of constancy between years (and months) is probably reasonable. Even if there were major differences in quantities between years, it is unlikely that individual sites (i.e. habitat types) would be differentially affected. This same assumption can be applied to relative tadpole densities. Observations at LTC beginning in 1975 and observations at numerous other localities in the Cascades indicate that areas in which tadpoles were concentrated were constant from year to year even when absolute abundances were not.

## Results

A summary of benthic sample analyses (Table 7) shows the substrate type samples were collected from and mean perimeter site densities for Bufo and Hyla (from field data of 1976). (Rana were present in sites A and D only in 1975 and 1976.) Substantial variability is apparent in the quantities of measured organics and chlorophyll both within and between sites. Large within sample variability (coefficients of variation for organics ranged from 0.7 to 100.3 with a mean of 45.3) is likely attributable in part to sampling technique. Imprecise replicate sampling, in volume sampled and surface depth of substrate penetrated, was probably responsible for much of this variability. A change in sampling technique or a larger replicate sample size might have resolved this problem. Differences in magnitudes of estimates, however, are generally larger between perimeter sites than within indicating these methods are sensitive to major differences in habitat. A one-way analysis of variance on 61 samples (organics) collected from mud and coarse substrates (Table 8) shows a highly significant substrate effect. Mud substrates were higher in total organics than coarse substrates.

As a relative estimate of the degree of association between species densities and organics, product-moment correlation coefficients were calculated. Values from each sample were used, not means. Coefficients ( $r$ ) for Bufo and Hyla were 0.92 ( $N = 61$ ;  $P < .001$ ) and 0.85 ( $N = 61$ ;  $P < .001$ ), respectively. High levels of organics and tadpole densities were predominately over soft substrates. There was also a significant



Table 7. Benthic sample analyses for total organics and chlorophyll a showing perimeter site of collection, substrate type, and mean tadpole densities at perimeter sites (taken from 1976 field data). FP = fine particulate substrate, S = sand, G = gravel, and R = rock. Asterisks (\*) indicate means comprised of two subsamples; all other means are of three samples.

Perim. Site	Substrate	Mean Species Density (No./ .56 m <sup>2</sup> )		Mean $\pm$ S.E.M. Organic Matter (mg volatile solids/l)	Chlorophyll <u>a</u> (mean ug/l)
		<u>Bufo</u>	<u>Hyla</u>		
A	Mud	30.2	5.6	1880.9 $\pm$ 217.88 6798.1 $\pm$ 2353.14 1405.6 $\pm$ 413.34*	929.0 $\pm$ 207.61*
B	Sand, Gravel	12.9	2.7	1159.4 $\pm$ 266.00 324.8 $\pm$ 230.32*	84.5 $\pm$ 35.14*
C	Mud	47.2	9.4	2413.2 $\pm$ 303.70 4706.8 $\pm$ 22.48* 8281.5 $\pm$ 707.18*	1245.8 $\pm$ 276.80*
D	Mud	25.3	5.5	2907.7 $\pm$ 564.46 2846.3 $\pm$ 513.44 1563.0 $\pm$ 73.04	1981.3 $\pm$ 266.75*
E	Mud	45.8	7.0	8032.7 $\pm$ 1995.24 5413.6 $\pm$ 2640.42	
F	Sand, Grav., Rock	7.3	0	419.3 $\pm$ 197.54 165.2 $\pm$ 76.12*	103.9 $\pm$ 55.62*
G	Mud, Sand	21.3	4.6	1904.3 $\pm$ 629.06 1049.9 $\pm$ 167.42*	537.6 $\pm$ 13.65*
H	Sand, Gravel	22.6	2.1	490.1 $\pm$ 124.58*	155.1 $\pm$ 0.59*
I	Sand, Grav., Rock	1.8	0	66.8 $\pm$ 27.64 195.4 $\pm$ 138.54*	
J	Mud, Sand	10.8	0.4	682.2 $\pm$ 131.00 2561.9 $\pm$ 832.42 675.5 $\pm$ 82.98	236.5 $\pm$ 38.97

Table 8. One-way analysis of variance of total organics (log transformed) in 36 benthic samples collected from mud substrates and 25 samples collected from coarse substrates.

Source	df	SS	MS	F	P
Substrate	1	12.998	12.998	85.5	< .001
Error	59	8.957	0.152		
Total	60	21.855			

association between organic content and chlorophyll content of a sample ( $r = 0.51$ ;  $N = 17$ ;  $P < .05$ ). Highest levels were generally associated with the finer substrates and low levels with coarse substrates.

Pigment analyses showed a considerable quantity of pheophytin was present in all samples (a mean of 34% of pigment content). Some degradation of chlorophyll could have occurred subsequent to collection and prior to analysis or these products may have been present at the time of collection. Nevertheless, interpretations regarding relative quantities of chlorophyll between sites are not seriously affected by this result, as the proportions of pheophytin are quite constant between samples. Optical density ratios (before/after acidification) ranged from 1.30 to 1.55, with a mean of 1.44.

These results suggest that substrate type could be used by tadpoles as a proximate indicator (cue) of food abundance. Mud substrates, where tadpoles spent most of their time, were richer in quantities of organics and chlorophyll than coarse substrates where densities were lowest.

## LABORATORY STUDIES

Field studies implicated many factors (temperature, depth, vegetation, substrate, shore distance, social factors, food) to be associated with tadpole distributions. Statistical analyses of these data, however, do not allow one to distinguish between variables merely correlated with tadpole distribution and those that are actually perceived and responded to by tadpoles. I used laboratory choice experiments to help determine the role of specific factors in habitat selection and the effect of rearing regime on habitat selection behavior.

### Substrate Preferences

Field distributions of tadpoles were strongly correlated with substrate type. Larvae were almost exclusively recorded over fine particulate substrates (mud) as opposed to coarse substrate types (sand, gravel, and rock). These experiments were designed to examine the ontogenetic development of substrate preference behavior in each species. Tadpole groups were reared from embryonic stages, or immediately following hatching, over one substrate only through development. During development groups from each treatment were tested (singly) periodically for substrate preference. Thus, the effect of substrate rearing experience and age and developmental stage on choice behaviors was examined.

## Methods

Test Animals. Rana larvae were propagated from adults in May 1977 and March 1979. Adults of 1977 were collected at LTC and those of 1979 from Green Lake and Satan Creek. Adults were collected from their respective populations each Fall (September–October) and maintained prior to ovulation induction in containers (26 x 19 x 10 cm) filled to a depth of 1–2 cm with tap water and kept in a dark environmental chamber at 2–4°C. Efforts to obtain Rana adults in Fall 1977 were unsuccessful (probably due to drought conditions that year). Wild-caught tadpoles tested for preferences in 1979 were collected from the same populations as adults of that year.

Methods for the induction of ovulation and fertilization of ova were adapted from procedures reported by Rugh (1948) and Haertel and Storm (1970). To obtain ova, parents were removed to 16°C 2 h prior to the injection of females with pituitary extract (Carolina Biological Supply). At first injection, each female received the equivalent of three pituitary glands. After 24 h, females were given a second intraperitoneal injection of one pituitary. If ovulations were successfully induced, ova were ripe for stripping approximately 40 h subsequent to the first injection. At this time, males were sacrificed and their testes macerated in 15 ml 0.1 N Holtfreter's solution. Fertilization of freshly stripped ova with sperm suspension took place in shallow, sterile petri dishes containing a small amount of 0.1 N Holtfreter's solution. After 1 h, eggs were then flushed and emersed in an aquarium of about 8 l of fresh 0.1 N Holtfreter's. This solution was

replaced with dechlorinated tap water 24 h later. At hatching, clutches were mixed and distributed across rearing habitats.

In the 1977 experiments (Rana series I), two male frogs were used to fertilize four females collected from LTC. From these four clutches, nearly 1,200 viable larvae were obtained. I placed 225 eggs in each of the four substrate rearing tanks about 1 day prior to hatching. Approximately equal proportions of the four clutches were allocated to all tanks to ensure mixing of progeny.

In the 1979 experiments (Rana series II), two males and three females collected at Satan Creek gave three egg clutches. Four of six Green Lake females produced eggs, which were fertilized by three males. These seven clutches produced approximately 1,700 viable larvae. Prior to hatching about 300 eggs were placed in each of the four rearing regimes. All clutches were mixed before placement except group N. Larvae of this group from Satan Creek and Green Lake were kept separate and tested as distinct groups through day 23.

In both years, all Rana groups were tested at three different periods during development. These periods generally corresponded to early, middle, and late developmental stages. Early tests were conducted very soon after the eyes were fully developed and swimming and feeding activity had begun. Late tests of larvae occurred prior to the final metamorphic stages and before both forelimbs had emerged and tail resorption commenced (before stage 42).

Hyla tadpoles were reared from embryonic stages collected from two sources. In March 1978, several clutches (numbering approximately 600 eggs) of similar developmental stage were obtained from a temporary pond

in Findley Wildlife Refuge, Benton County, Oregon. Several clutches comprising about 300 eggs were collected in June 1979 from LTC. All wild-caught tadpoles came from the Green Lake population and were tested in August 1978 and October 1979. In the 1978 experiment each larval group was tested once early and once late in development. In the 1979 experiment, tadpoles were tested once late in development. Test substrate pairs always included the substrate over which a group was reared.

Bufo larvae were from three newly oviposited clutches obtained from LTC in June 1978. Tadpoles were tested for preference once, late in development. Wild tadpoles were collected in October 1979 from Todd Lake.

Apparatus. Three aquaria, used for the rearing and testing of all groups, were constructed of 19 mm thick plywood and painted and sealed with gray epoxy paint (Pratt and Lambert). Inside dimensions of one of the tanks was 117 x 57 x 61 cm and the remaining two were 117 x 57 x 45 cm. Tanks were situated on a bench 128 cm beneath (measured from the water surface) a bank of three-bulbed 40W, cool-white lights. Air temperature was maintained between 16 and 21°C during rearing and a light cycle of 16L:8D was imposed. Each tank was filled to a depth of 20 cm with approximately 100 l of dechlorinated tap water and aerated with a single air stone. Tanks were mostly drained and replaced with freshwater every 7-12 days. Larvae were fed rabbit pellets once daily. One tank was partitioned into three compartments (representing three substrate rearing regimes) using 2 mm thick opaque plastic sheets



of the dimensions 57 x 45 cm and painted gray. The test tank was partitioned into halves so that two choice experiments could be carried out simultaneously.

Substrates. The primary objective in selecting test substrates was to approximate as closely as possible types naturally available to these species in the high lakes, and particularly those available at LTC. The criteria used were grain size (coarseness) and texture. To assure substrate uniformity, constancy of grain size, and biologic "sterility", three substrates were obtained from a sand and gravel supplier. Their texture and grain size closely corresponded to the spectrum of common substrate patch types available along the shore terrace of LTC. The substrates and their grain diameters were sand (<2 mm), gravel (4-8 mm), and rock (15-35 mm). The surface textures of the gravel and rock were smooth and rounded.

A dominant class of substrates not represented by these is the very fine particulate, muddy substrates. It was not practical to reproduce this type in the lab without introducing several unwanted factors (e.g. food material and turbidity). Therefore, bare tank bottom, which lacks any significant grain component, represented the fourth and smallest grain substrate tested. Substrate types are henceforth abbreviated as N (bare tank), S (sand), G (gravel), and R (rock).

Procedure. Half of each test compartment was assigned to one substrate type. Tadpoles were placed singly into a 10-cm diameter Plexiglas cylinder, open at both ends and situated vertically in the center of the compartment. After 5 min and when the tadpole was motionless on the

bottom, orientation within the release chamber was noted. The chamber was then gently lifted to the top of the tank using an external monofilament line, and the initial substrate choice recorded. Initial choice is a good predictor of preference in tadpoles (Wiens 1970, 1972; O'Hara 1974). Habitat choice was defined as the substrate to which initial movement was directed and over which the tadpole penetrated a minimum of 10 cm perpendicular distance from the substrate edge. If a choice was not made within 5 min, the tadpole was removed and another tested. At each test the developmental stage of a test group was estimated by staging a minimum of 10 individuals from the group according to Gosner (1960). In the 1979 tests of Rana, some individuals from the second test series were retained and used in the late experimental series. None of these tadpoles, however, was tested more than twice. All other tadpoles were tested once.

At capture, wild tadpoles were kept in a clean translucent white container until testing was completed. Groups were tested for substrate preferences within 3 days of capture; all individuals were tested one.

The null hypothesis under test was that substrate type had no effect on choice behavior and that the frequencies of substrates selected by a group would therefore not differ significantly from a random distribution. The binomial test (Sokal and Rohlf 1969) was used to calculate the probability of obtaining the observed distribution given an expected equal distribution of choices between substrates (two-tailed). If a calculated cumulative probability was less than or equal to 0.05,  $H_0$  was rejected.

## Results

Rana. A summary of results (Table 9; for a complete listing of results see Appendix IV) shows the major patterns in substrate preference. Analyses of pre-release orientations (Appendix IV) show that this criterion was a poor predictor of preference, although trends were in favor of the preferred substrate (but not significant). Initial choices, however, were clearly interpretable. Regardless of group experience, group age, or year of the experiment, larvae tended to prefer the substrate of finer grain size in any single paired combination. Substrate N was most preferred, while R was least preferred. In only one of 107 tests performed was the coarser substrate preferred (R over G by group R in the final test of series I).

Choices were not always clearly exhibited. In 31 tests, no preference was indicated. It is notable, however, that of the substrate pairs offered in these tests 27 involved pairs of "similar" grain sizes (N:S, S:G, and G:R). This suggests that such pairings were not as clearly distinguished by tadpoles as more contrasting substrate types.

These data also suggest some dependence of substrate selectivity on tadpole age or developmental stage. Of the 31 tests exhibiting no preference, 16 occurred in the 36 tests performed prior to day 29, 8 between days 39 and 59 (30 tests), and only 6 later than day 67 (40 tests). Eight of 10 groups tested within the first 15 days of larval life showed no preference (the two exceptions were tests N:G and N:R). Substrate preferences, or perhaps perceptual capabilities, may not be as clearly differentiated in very young tadpoles. However, there is no

Table 9. Summary of 107 substrate preference tests with Rana tadpoles (ages 8 to 111 days).

Substrate Choices	No. Tests	No. Tests in which <u>Finer</u> Substrate was Selected	No. Tests in which <u>Coarser</u> Substrate was Selected
Bare : Sand	18	7	0
Bare : Gravel	18	15	0
Bare : Rock	17	17	0
Sand : Gravel	18	9	0
Sand : Rock	18	17	0
Gravel : Rock	18	10	1

other evidence to suggest that substrate preferences are dependent on age or developmental stage in Rana tadpoles.

The high consistency in choice behaviors across groups exhibited by these larvae indicates that substrate experience, even over the long term, had little influence on the manifestation of the behavior. There were perhaps two exceptions. Both occurred in R tadpoles tested for S:R and G:R. In test S:R, every R group tested at all ages of both years selected S except 83-day-old, series I tadpoles, which showed no preference. In test G:R, R individuals from both series changed in their responses to R in the last test. This suggests that lengthy experience with R was sufficient to change but not reverse early responses to that substrate, provided substrate pairs were not extremely different in type (no change with time was evident in tests of N:R for these groups). The lack of a consistent trend however, suggests that experience contributes little to substrate preferences in Rana tadpoles.

These data also allow comparison of substrate selection behavior between tadpoles of different natural populations. In one case, a subsample of each of the two progeny groups used in experimental series II was isolated for a time in habitat N during early development. Results of independent tests performed on these groups indicate similarity among all tests in choice distributions (Table 10). Because experimental procedures were identical in both years, it is also possible to compare behavior of progenies of each series. Progeny of series I were from parents collected at LTC and in series II from Satan Creek and Green Lake. Direct comparisons (see Appendix IV) can be made

Table 10. A comparison of substrate choice distributions of Rana progeny from two populations.

Green Lake			Satan Lake		
Age (Days)	Initial	Choices	Age (Days)	Initial	Choices
8	$\frac{N}{19}$	$\frac{S}{11}$	11	$\frac{N}{11}$	$\frac{S}{19}$
11	$\frac{N}{11}$	$\frac{G}{4}$	11	$\frac{N}{13}$	$\frac{G}{2}$
17	$\frac{S}{10}$	$\frac{G}{2}$	17	$\frac{S}{11}$	$\frac{G}{2}$
19	$\frac{S}{9}$	$\frac{R}{1}$	19	$\frac{S}{10}$	$\frac{R}{1}$
23	$\frac{G}{9}$	$\frac{R}{1}$	23	$\frac{G}{9}$	$\frac{R}{1}$

with 12 pairs of series I and II test groupings. Of these 12 pairs, eight show very close correspondence in results between series. There is no evidence to suggest that population source affects substrate selection behavior.

Hyla. Hyla tadpoles from two populations (Table 11) also preferred finer grained substrates. In no case was a coarser substrate type preferred. Rearing regime or age had no apparent influence on preference. Substrate pairs bearing the greatest similarity (N:S, S:G, and G:R) were not as clearly distinguished as more dissimilar pairs.

Bufo. Of 12 tests performed with Bufo tadpoles, eight distributions of initial choice were not statistically different from random (Table 12). The four tests that were significant, were biased toward the finer substrate and involved substrate pairs of widely divergent types (N:G, N:R, and S:R). A coarser substrate was never preferred by a test group and when choices are summed across all groups, 155 tadpoles selected finer grained substrate and 85 coarser substrates (significant at  $P < 0.02$ ).

Wild-caught Tadpoles. Substrate preferences of wild tadpoles (Tables 13, 14, 15) were similar to lab-reared larvae. Finer substrates tended to be preferred (see combined totals) even though few individual tests were significant.

One possible confounding factor in the use of these substrate types is that each one differed in reflective light intensity. There was a decrease in lightness with increasing substrate coarseness (N was

Table 11. Results of substrate preference tests with lab-reared Hyla from Willamette Valley and LTC populations.

Group	Age (Days)	Initial Choices	
		<u>Bare</u>	<u>Sand</u>
Bare	10	17	3*
Bare	34	14	6
Bare†	36	13	7
Sand	10	11	9
Sand	34	12	8
Sand†	38	12	8
		<u>Bare</u>	<u>Gravel</u>
Bare	11	17	3*
Bare	35	18	2*
Bare†	36	18	2*
Gravel	11	16	4*
Gravel	35	15	5*
Gravel†	38	16	4*
		<u>Bare</u>	<u>Rock</u>
Bare†	35	15	5*
Rock†	34	18	2*
		<u>Sand</u>	<u>Gravel</u>
Sand	12	12	8
Sand	36	17	3*
Sand†	35	13	7
Gravel	11	10	10
Gravel	36	13	7
Gravel†	34	16	4*
		<u>Sand</u>	<u>Rock</u>
Sand†	39	16	4*
Rock†	40	14	6
		<u>Gravel</u>	<u>Rock</u>
Gravel†	39	11	9
Rock†	40	13	7

\*  $P < .05$

† LTC tadpoles



Table 12. Results of substrate preference tests with lab-reared Bufo.

Group	Age (Days)	Initial Choices	
		Bare	Sand
Bare	48	<u>12</u>	<u>8</u>
Sand	50	9	11
Bare	48	<u>16</u>	<u>Gravel</u>
Gravel	50	13	4*
Bare	56	<u>16</u>	<u>Rock</u>
Rock	59	17	4*
Sand	53	<u>14</u>	<u>Gravel</u>
Gravel	54	12	6
Sand	56	<u>15</u>	<u>Rock</u>
Rock	59	13	5*
Gravel	53	<u>10</u>	<u>Rock</u>
Rock	54	8	10
Total		<u>155</u>	<u>85*</u>

\*  $P < .05$

Table 13. Substrate preferences of wild Rana tadpoles.

Source	Dev. Stage	Initial Choices	
Todd L.	36	<u>Sand</u> 12	<u>Gravel</u> 8
		<u>Sand</u> 18	<u>Rock</u> 2*
		<u>Gravel</u> 19	<u>Rock</u> 1*
Green L.	33	<u>Sand</u> 10	<u>Gravel</u> 10
		<u>Sand</u> 14	<u>Rock</u> 6
		<u>Gravel</u> 19	<u>Rock</u> 3*
Total		<u>92</u>	<u>30*</u>

\* P &lt; .05

Table 14. Substrate preferences of wild Hyla tadpoles.

Source	Dev. Stage	Initial	Choices
Green L.	33	<u>Sand</u> 13	<u>Gravel</u> 7
		<u>Sand</u> 13	<u>Rock</u> 7
		<u>Gravel</u> 15	<u>Rock</u> 7
Green L.	36	<u>Bare</u> 11	<u>Sand</u> 9
		<u>Bare</u> 18	<u>Gravel</u> 2*
		<u>Bare</u> 16	<u>Rock</u> 4*
		<u>Sand</u> 14	<u>Gravel</u> 7
		<u>Sand</u> 19	<u>Rock</u> 6*
		<u>Gravel</u> 14	<u>Rock</u> 8*
		Total	

\* P &lt; .05

Table 15. Substrate preferences of wild Bufo tadpoles.

Source	Dev. Stage	Initial	Choices
Todd L.	37	<u>Bare</u> 17	<u>Sand</u> 5*
		<u>Bare</u> 13	<u>Gravel</u> 7
		<u>Bare</u> 10	<u>Rock</u> 10
		<u>Sand</u> 17	<u>Gravel</u> 9
		<u>Sand</u> 15	<u>Rock</u> 5*
		<u>Gravel</u> 11	<u>Rock</u> 9
Total		<u>83</u>	<u>45*</u>

\* P &lt; .05

lightest, R darkest). Some species of tadpoles are responsive to sharp differences in light intensities, and this could partially explain observed choice responses.

During testing in 1979, a standard testing tank was modified to test for tadpole responsivity to substrate lightness. On one half of the tank, a 0.5 cm thick sheet of black vinyl plastic was fastened to the substrate (bottom only). The opposing half represented substrate N (gray). Two groups of Rana tadpoles reared in habitat N were tested at days 29 and 30.

Results showed that responses of both groups were random in respect to substrate lightness and darkness. Of 40 tadpoles tested, 23 selected substrate N and 17 chose the black substrate. Because reflective intensities of these substrates differed more than those among the experimental substrates, I concluded that reflective intensity was not a significant variable affecting substrate selection.

## Vegetation Preferences

Tadpole distributions in the field were associated with distributions of Carex and Isoetes. Carex, particularly, was a significant element of Rana and Hyla habitats at most localities surveyed. Clumps of Carex were simulated in the laboratory and tadpoles were tested for preference between an "open" (N) and vegetated (V) habitat. Artificial vegetation was used to represent the visual image of Carex in the absence of possible olfactory or food cues that might be associated with real vegetation.

### Methods

Test Animals. Experimental tests with Rana were carried out simultaneously with substrate tests of 1977 (LTC Rana). Wild Rana were collected from Todd and Green Lakes in 1978. Lab-reared Hyla were of the 1978 experimental group and wild tadpoles were collected from Green Lake in 1978. Lab-reared Bufo were of the same experimental group collected from LTC in 1978. Wild Bufo came from Todd Lake.

Apparatus and Procedure. Two aquaria were used for the conditioning and testing of tadpoles. Tank N was used to represent an open, non-vegetated habitat. Tadpoles conditioned with "vegetation" (group V) were placed within a compartment of another tank. Compartment size was 39 x 19 x 61 cm, one-third the size of the standard tank. The remaining two-thirds of the tank served as a test tank for N:V tests. In the 1977 test series, 70 Rana tadpoles were placed at hatching in habitat V; and

in 1978, 60 Hyla tadpoles were placed at hatching in habitat V.

Sixty Bufo tadpoles were placed in a compartment of habitat V in 1978.

Clumps of simulated vegetation placed in the tanks were designed to resemble Carex visually. Thirty-two strips of green vinyl plastic comprised each clump. Each strip was 2 mm wide and 20 cm in length. Strips were fastened together at their base and anchored in an upright position to the tank bottom with silicone rubber sealer. Clumps were arranged in a uniform pattern (Figure 5). Conditions of rearing and testing were identical to those described for substrate experiments. No tadpole was tested more than once.

## Results

Results of choice experiments for each species (Table 16) show a tendency by tadpoles to prefer open habitat over "vegetated" habitat. While preferences for open were not consistently evident between tests (e.g., in no single test was a preference exhibited by Hyla tadpoles), combined choice distributions for each species were significant. Rearing experience had no effect on choice.

## VEGETATION PREFERENCE TANK (Top View)

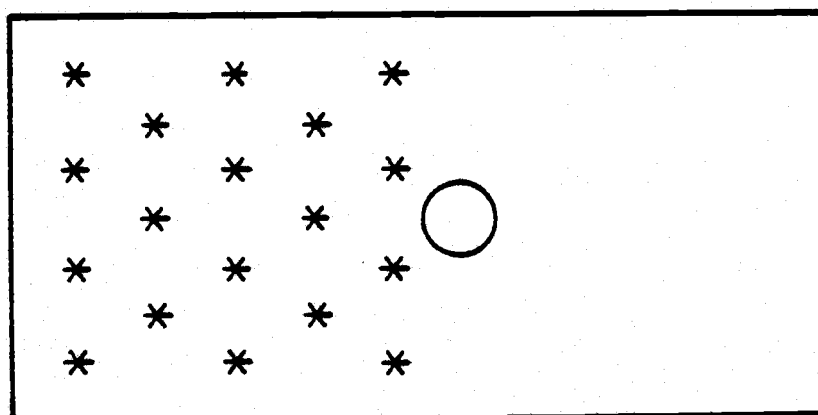


Figure 5. Test tank used to test preferences of vegetated vs. open habitat. Inner circle denotes tadpole release point.



Table 16. Vegetation - open habitat preferences of Rana, Hyla, and Bufo tadpoles. Wild tadpoles were collected from Todd Lake (T.L.) and Green Lake (G.L.).

Species	Group	Dev. Stage	Initial Choices		P
			Open	Vegetation	
<u>Rana</u>	Open	26	19	1	.001*
	Veg.	26	19	1	.001*
	Open	36	16	8	.152
	Veg.	36	16	9	.230
	Open	38	10	10	
	Veg.	39	13	7	.263
	Wild(T.L.)	36	16	4	.012*
	Wild(G.L.)	35	<u>13</u>	<u>7</u>	<u>.263</u>
	Combined		122	47	.001*
<u>Hyla</u>	Open	26	13	7	.263
	Veg.	26	14	6	.115
	Open	37	12	8	.503
	Veg.	38	9	11	.824
	Wild(G.L.)	35	<u>13</u>	<u>8</u>	<u>.383</u>
	Combined		61	40	.046*
<u>Bufo</u>	Open	35	14	6	.115
	Veg.	36	15	5	.041*
	Open	35	12	8	.503
	Veg.	35	10	10	
	Wild(T.L.)	37	21	9	.043*
	Wild(T.L.)	37	<u>16</u>	<u>9</u>	<u>.230</u>
	Combined		88	47	.001*

### Social Factors: Aggregation Tendencies

Social factors (i.e., the presence of conspecifics) could be important variables affecting tadpole distributions and the habitat selection process. In the present study, field distributions of each species were highly contagious. While aggregations took on a variety of forms, (i.e., size and density), individual tadpoles were nearly always found nearby other conspecifics. Whether tadpoles were responding to each other or to some common environmental cue was not possible to discern from field observations alone. Therefore, I tested in the laboratory the possible role of mutual social attraction in producing these clumped distributions.

### Methods

An apparatus was constructed similar to that used by Wassersug and Hessler (1971) and Wassersug (1973) in testing aggregative tendencies of several species of tadpoles. A tank of the dimensions 55 x 55 x 18 cm was partitioned into four equal compartments using 5 mm thick clear Plexiglas. Each compartment was further subdivided into quadrants by lightly penciling in lines on the bottom of the tank (Figure 6). By placing one tadpole in each of the four compartments and recording its quadrant locations through time, a measure of visually mediated social attraction could be obtained. If individuals spent most of their time in quadrant 1, less in quadrants 2 and 3, and least in 4, then the tendency to aggregate would be strongly positive. If the greatest

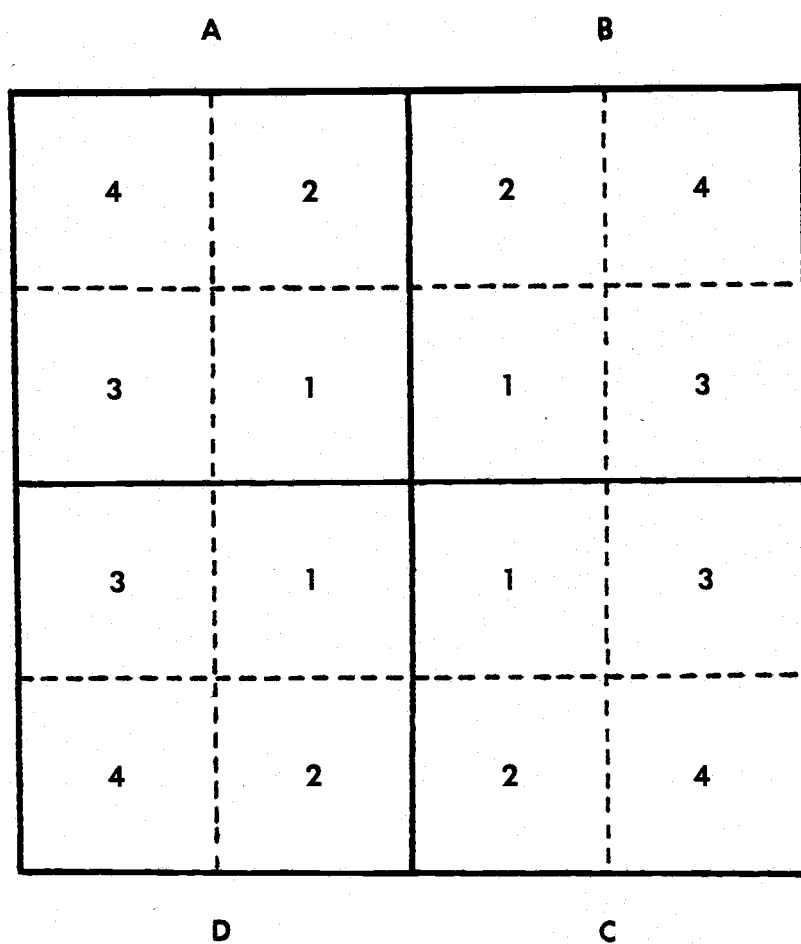


Figure 6. Apparatus (top view) used to test tadpole aggregation tendencies. One tadpole was placed in each quadrant (A-D).

proportion of time was spent in quadrant 4 and the least in 1, then mutual avoidance would be indicated.

After each of four tadpoles was placed in their respective compartments, a period of 10 min was allowed to elapse before the first observation was taken. I recorded quadrant positions at 30 or 60 s intervals (depending on the test), but not until each individual had moved at least 5 cm in distance since the previous observation. This latter condition was required to insure independence between observations. A tadpole was considered in a quadrant if its eyes were positioned within the quadrant, even if the rest of its body was not. Test length varied with tadpole activity and time constraints on the observer. Tank water was changed following each test. Control tests consisted of placing one individual in only one of the four compartments.

Frequency distributions were tested for goodness of fit using the G-test (Sokal and Rohlf 1969). Expected frequencies based on a random distribution for each quadrant were .25.

All lab-reared tadpoles used in tests of aggregation tendencies were of group N. Rana tadpoles were taken from 1977 and 1979 lab groups and wild tadpoles from Green Lake. Hyla larvae were from 1978 and 1979 lab groups and wild tadpoles from Green Lake. Bufo were taken from the 1978 lab group and LTC and Todd Lake wild populations.

## Results

Rana. Quadrant distributions of two control tests run with single tadpoles (Figure 7) were not significantly different from random. There

*Aggregation Experiments*  
*R. cascadae (Control Tests)*

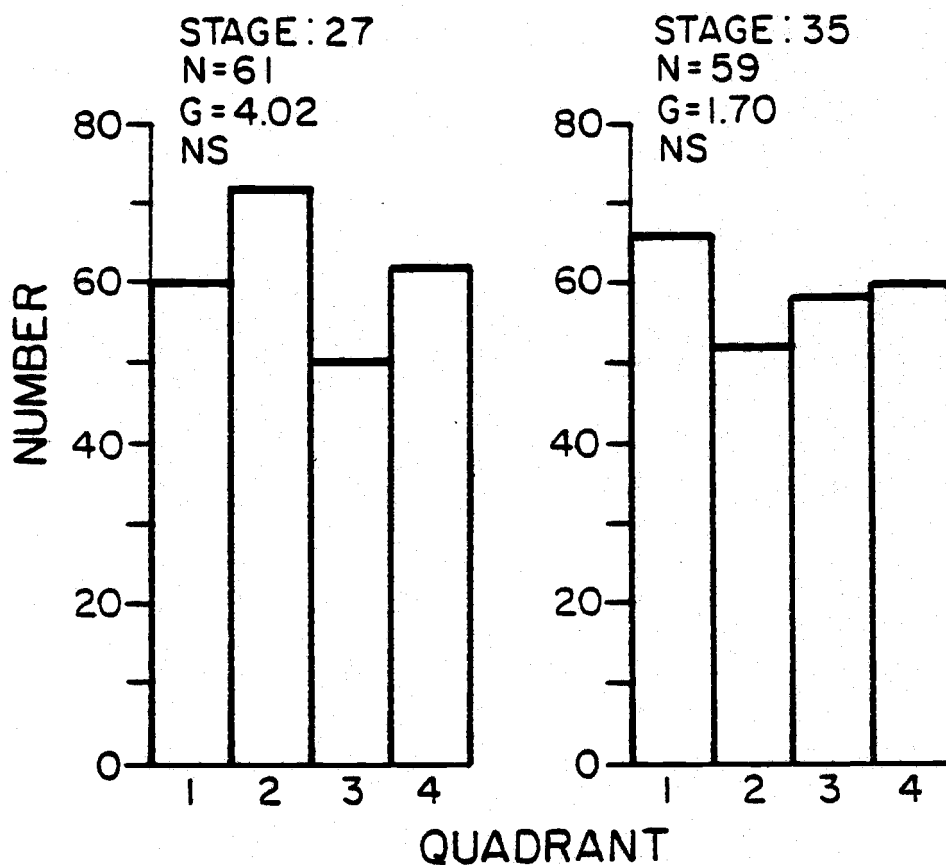


Figure 7. Results of Rana aggregation control tests (one tadpole each test) showing developmental stage, number of observations (N), and G-statistic.

were apparently no biases inherent in the design of the test apparatus. Experimental tests were staged on seven occasions (Figures 8 and 9). For all tests, frequency distributions across quadrants varied significantly from random. With the exception of one test (Figure 8), distributions favored central quadrant 1. Quadrant 4 was least preferred. Wild tadpoles (Figure 9) behaved as lab-reared ones and no difference was evident between larvae of different developmental stage. The single inconsistent distribution can be attributed primarily to the behavior of one individual that favored quadrants 2 and 4.

Hyla. Comparable results were obtained in tests with Hyla larvae and are presented in Figure 10. Data from the control test were random in distribution. Three tests showed high aggregative tendencies similar in distributions to those recorded for Rana. One test did not fit the hypothesized distribution for aggregation.

Bufo. Bufo tadpoles also showed a strong tendency to aggregate (Figure 11). One control test was random in distribution and all three experimental tests exhibited a distribution favoring the central quadrant.

These data support the hypothesis that Rana, Hyla, and Bufo tadpoles possess aggregative tendencies and that these behaviors can be elicited by visual stimuli alone.

# Aggregation Experiments

## R. cascadae (Lab)

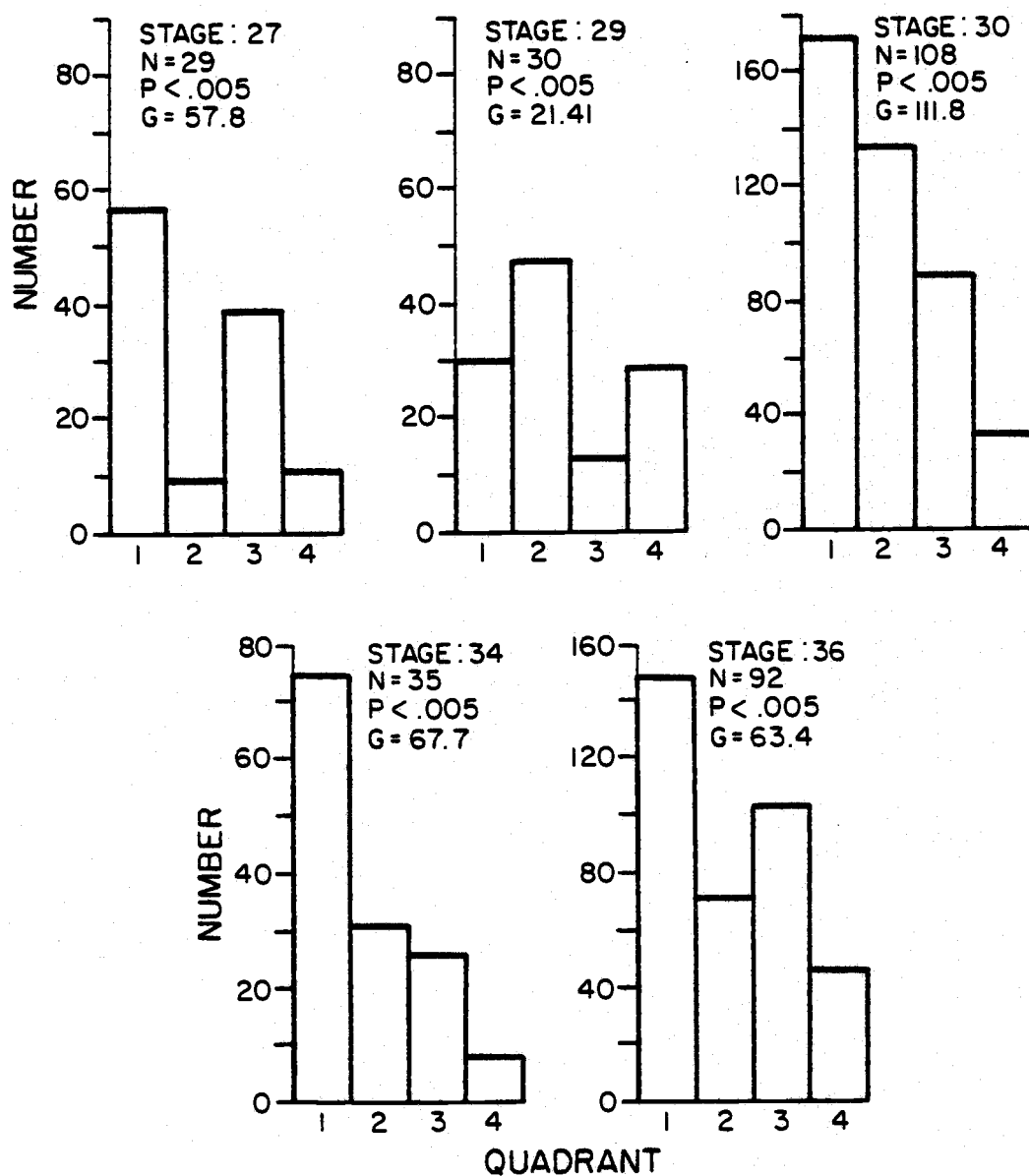


Figure 8. Results of *Rana* aggregation tests (four lab-reared tadpoles each test) showing tadpole quadrant distributions, developmental stage, number of observations (N), and G-statistic.

*Aggregation Experiments*  
*R. cascadae* (Wild)

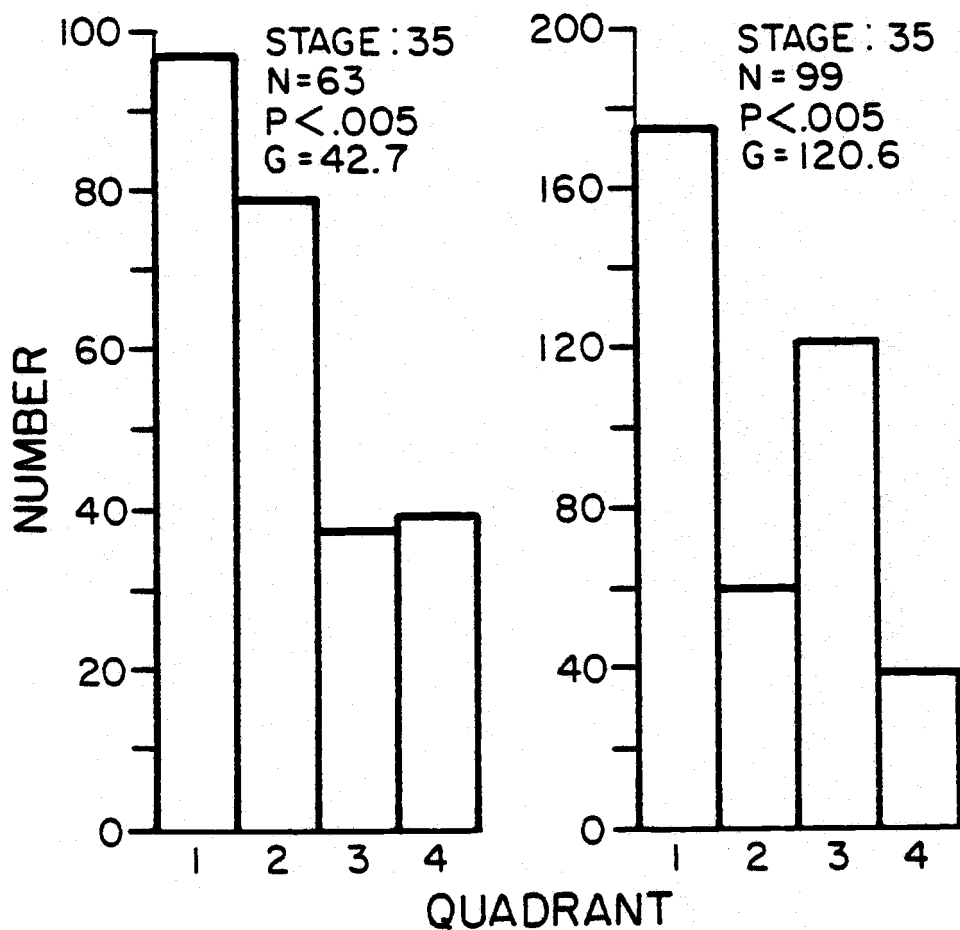


Figure 9. Results of *Rana* aggregation tests (four wild tadpoles each test) showing tadpole quadrant distributions, developmental stage, number of observations (N), and G-statistic.



*Aggregation Experiments*  
*Hyla regilla*

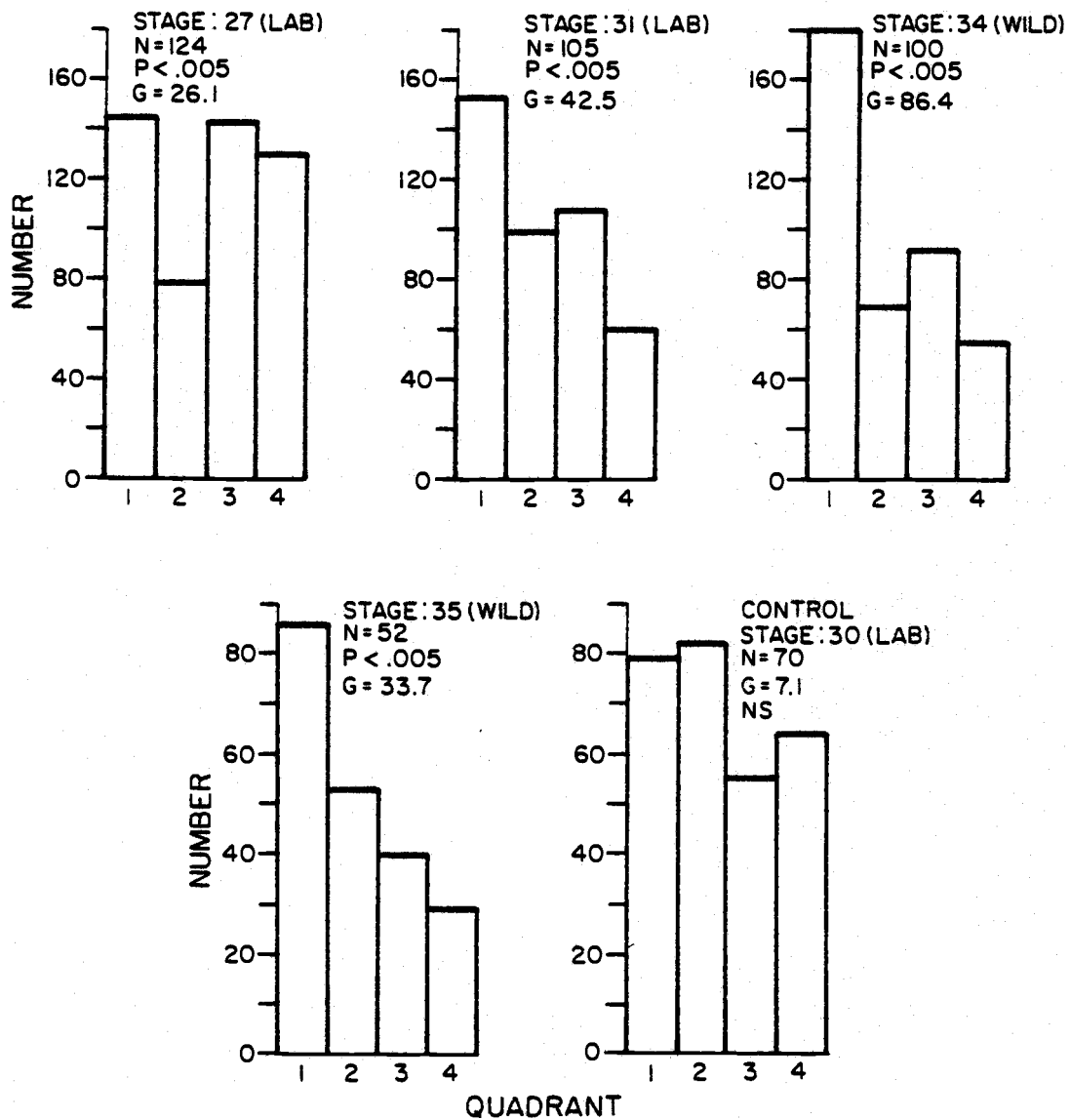


Figure 10. Results of *Hyla* aggregation tests (four tadpoles each test except control) showing tadpole quadrant distributions, developmental stage, number of observations (N), and G-statistic.

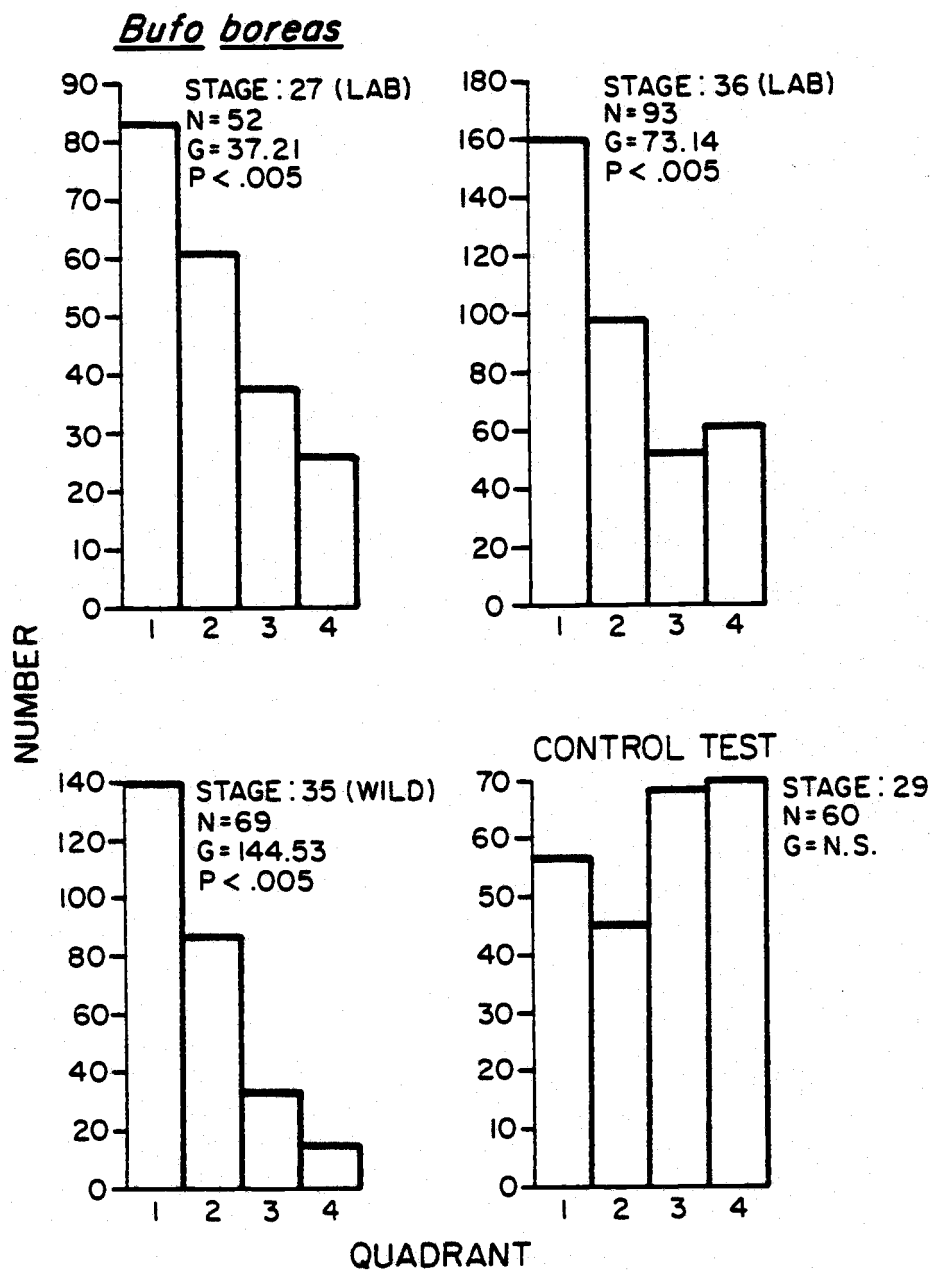


Figure 11. Results of Bufo aggregation tests (four tadpoles each test except control) showing tadpole quadrant distributions, developmental stage, number of observations (N), and G-statistic.

## Water Depth

Water depth was negatively correlated with tadpole densities in the field. But depth itself was correlated with other variables, namely temperature, distance from shore, vegetation, and perhaps food. To help distinguish the role of depth in habitat selection, a laboratory test was designed to test responses to this variable in the absence other confounding factors.

### Methods

A tank (117 x 57 x 61 cm) was modified to test for depth preferences by inserting panels to create three discrete depths (Figure 12). Two groups each of Rana and Hyla were tested in this apparatus in 1979, however, tests with Hyla were unsuccessful due to inactivity in the test apparatus. One group of Rana was reared for 89 days at a depth of 5 cm in a featureless tank (N). A second group was reared in the same way for 91 days at a depth of 59 cm. One group of wild Bufo from Todd Lake was tested.

In each test bout two individuals of the same species were tested simultaneously. Each individual was released over one of the three depths such that no one depth was favored as a release point for any group. Tadpoles were allowed to acclimate for 15-20 min prior to the start of time recording. Times spent in each depth section were recorded in seconds until 15 min total time had elapsed for each individual. During each test, if a tadpole was inactive for more than

## DEPTH PREFERENCE TANK (Side View)

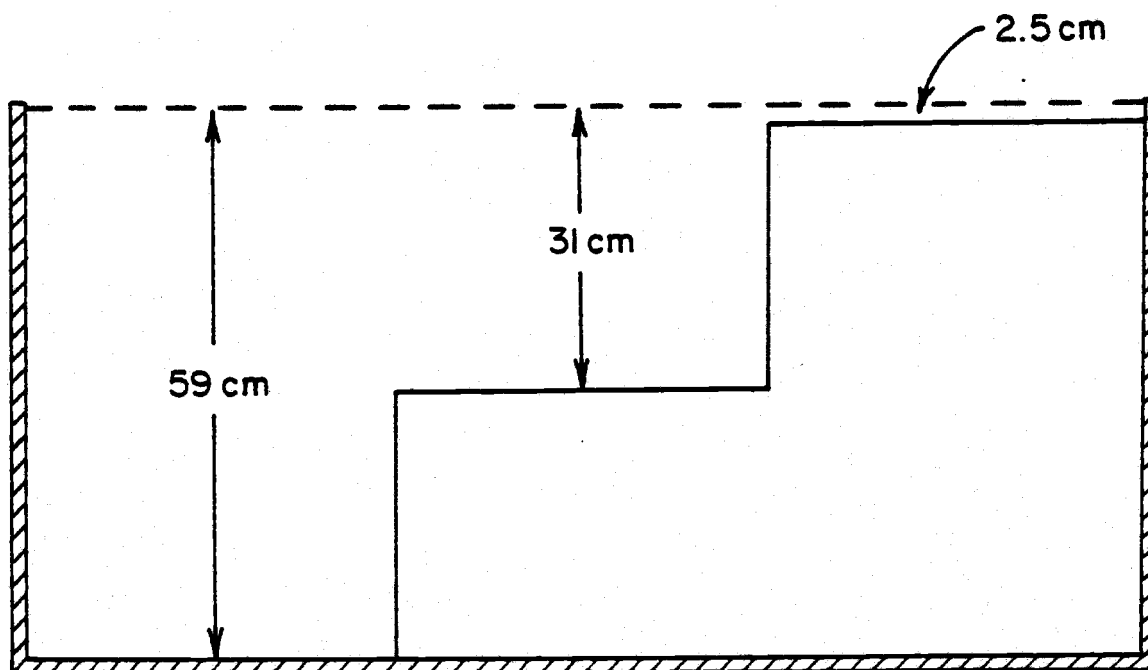


Figure 12. Side view of tank used to test tadpole water depth preferences.

30 s, time was stopped until activity was resumed. Following each test, water was mixed and fecal material removed.

Expected values of time spent over each depth were calculated in respect to total space, or water volume, available at each depth. Therefore, a tadpole moving randomly through the tank for a 15-min period would be expected to spend 24 s at 2.5 cm, 302 s at 31 cm, and 574 s at 59 cm. Time distributions for each group were subjected to a one-way analysis of variance. For each tadpole, expected times were subtracted from observed times. A constant was then added to this value for each depth to avoid negative outcomes (530 for Rana groups; 300 for the Bufo group). Because variances in times differed with respect to depth, these data were log transformed prior to the analysis of variance.

## Results

Analyses of variance for both Rana groups (Table 17) show a highly significant depth effect. When mean times spent at each depth are compared with expected times (Table 17), a pronounced effect due to rearing regime is apparent. Tadpoles reared at 5 cm spent more time at 2.5 cm and less time at 59 cm than expected. Tadpoles reared at 59 cm spent less time at 2.5 cm and more time at 59 cm than expected. Tadpoles tended to prefer depths corresponding to their rearing environment.

The analysis of variance for the Bufo group (Table 18) also shows a significant depth effect. In this case, more time was spent at 2.5 cm and less time at 31 cm than expected. No difference was observed at

Table 17. Depth preferences and one-way analyses of variance of results of Rana tadpoles reared in shallow water (5 cm) and deep water (59 cm).

REARED AT 5 CM DEPTH

Depth (cm)	N	Observed Mean $\pm$ S.E. (sec)	Expected Mean (sec)
2.5	22	147.1 $\pm$ 35.87	24.3
31.0		373.6 $\pm$ 42.83	301.5
59.0		379.3 $\pm$ 50.10	574.2

Source	df	SS	MS	F	P
Depth	2	3.168	1.584	12.57	<.001
Error	63	7.957	0.126		
Total	65	11.125			

REARED AT 59 CM DEPTH

Depth (cm)	N	Observed Mean $\pm$ S.E. (sec)	Expected Mean (sec)
2.5	20	9.3 $\pm$ 5.75	24.3
31.0		231.3 $\pm$ 36.79	301.5
59.0		659.5 $\pm$ 37.13	574.2

Source	df	SS	MS	F	P
Depth	2	0.189	0.094	6.52	<.005
Error	57	0.824	0.014		
Total	59	1.013			

Table 18. Depth preference and one-way analysis of variance of results of wild Bufo tadpoles.

Depth (cm)	N	Observed Mean $\pm$ S.E. (sec)	Expected Mean (sec)
2.5	20	87.4 $\pm$ 22.42	24.3
31.0		222.1 $\pm$ 36.23	301.5
59.0		590.6 $\pm$ 37.59	574.2

Source	df	SS	MS	F	P
Depth	2	0.993	0.496	4.35	< .025
Error	57	6.467	0.114		
Total	59	7.460			

59 cm depth. It is not known why tadpoles would spend relatively less time at 31 cm than at 59 cm. But since Bufo tadpoles in the field ranged in deeper water than 59 cm, it is possible that this depth was not "aversive" in a tank of uniform temperature, food, etc.



### Entrainment Experiments: Multiple Factors

In the real world, organisms continually and simultaneously encounter many environmental stimuli. The important question of how different environmental factors interact in eliciting behavioral responses is difficult to answer using experiments that manipulate single factors. But once responses to single factors are assayed, those factors can be used in combination to help answer more complex questions about preference behaviors.

Through experimentation and field observation responses to three environmental variables by Rana tadpoles became known. Finer substrates were preferred over coarse substrates and responses were not influenced by experience. Higher water temperatures were selected over lower temperatures. Food, when present in discrete packets, was vigorously sought after by tadpoles and was correlated with substrate type in the field.

In these experiments, I was interested in the adaptability (flexibility) of substrate preference behaviors and how other factors might interact with substrate in affecting preference behaviors. Rana tadpoles were reared in complex environments containing two substrate types. During rearing, a positive stimulus (high temperature or food) was associated with the nonpreferred substrate (rock) and a "negative" stimulus (low temperature or no food) was associated with the preferred substrate (bare tank, N). Tadpoles were periodically tested for substrate preference during development. Similar conditions such as these could occur naturally if the preferred habitat becomes unsuitable

or dries up due to seasonal changes. Tadpoles whose choice behaviors became modified were tested for retention capabilities.

## Methods

One tank (122 x 40 x 30 cm) was partitioned in half longitudinally and used for the conditioning of the two experimental groups (Figure 13). Half of each compartment comprised substrate N, the remaining halves substrate R. Water was filled to a depth of 15 cm. In one compartment (experiment or group T), temperature was manipulated such that the water over substrate R was maintained 2.5°C higher than that over substrate N (room temperature). Heat was provided by an aquarium heater placed vertically at the extreme end of the tank. To aid in maintaining the gradient, a clear plexiglass partition was placed between the two substrates such that a space 2 cm high was open at the base. Single air stones positioned at each end kept water temperatures uniformly mixed. Food (rabbit pellets) was uniformly distributed over each side once daily.

In the second compartment (experiment or group F), temperature was kept uniform (18–21°C) and tadpoles were fed only over the rock substrate. Substrate N was cleaned twice daily of food and fecal material during the course of the experiment.

At 4 days posthatching, 130 tadpoles were placed in each experimental compartment. After varying lengths of exposure to these regimes, tadpoles were tested for preferences between substrates N and R (for test procedure see Methods section of Substrate Experiments) in a tank of uniform temperature and no food. Results of substrate choices

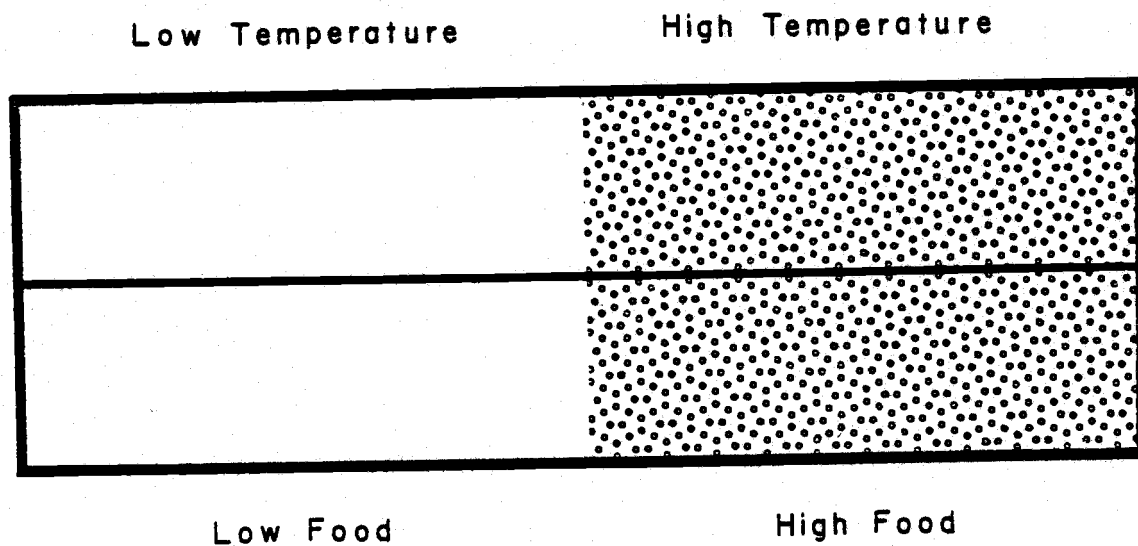


Figure 13. Diagram of entrainment tanks used to rear two groups of Rana tadpoles. One group experienced higher temperature over a rock substrate relative to a bare tank (N) substrate. A second group was given food only over the rock substrate.

were categorized as left or right in direction. This referred to the side of the test tank from the experimenter's point of view a particular substrate was placed and was utilized as a control measure for the test tank. Following entrainment, retention of learned behaviors (for group T only) was tested in two ways. One group (25 tadpoles) was left in the experimental tank with the heater off (i.e. the temperature gradient removed) and subsequently tested for preference. A second group (40 tadpoles tested on days 37 and 38 plus 20 untested tadpoles) was moved to a featureless tank in a different part of the lab on day 38 and later tested. For both experiments (T and F) any one test was completed in less than 1 day. Because numbers of tadpoles and space were limited, some individuals in experiment T (retention tests only) and in the last test of the control group (see below) were tested more than once. However, no individual was tested twice in less than 2 days. Tests at 1, 4, and 17 days experience (experiment T) were performed subsequent to other tests. These tadpoles were taken from a featureless rearing regime but were older and of a later developmental stage.

One control group of 75 tadpoles was founded at the same time as the experimental groups. This group was reared in a tank (58 x 57 x 61 cm) that was equally divided into substrates N and R. Control tadpoles were tested in the same tank and in the same manner as experimental groups.

## Results

During rearing of group T tadpoles, distributions were highly biased toward side R (i.e. higher temperature). This bias was quickly established within the first 2 h after tadpoles were placed in the

tank. At any one time during the experiment, approximately 80-90% of the tadpoles would be located over substrate R. Results, however, show that substrate preferences were unchanged for at least 7 days (Table 19), after the experiment was begun. Substrate N was preferred and choice behavior was indistinguishable from the control group. At day 30, the first change in substrate choice behavior was observed. Substrate R, previously avoided by tadpoles, was preferred over N. But in the test of day 37, tadpoles preferred substrate N over R. The only procedural difference in this test from that of day 30, was that the sides substrates were placed in the test tank were reversed (left to right). The importance of direction (left and right) was substantiated by the test at day 38 which utilized a completely uniform (N) tank. As in tests of days 30 and 37, the right hand side was preferred.

Because there were no biases in cues internal to the test tank at day 38, the only cues tadpoles could have been using were cues above the tank in the lab. Initially overlooked was the fact that these overhead cues (lights and cupboards) were not completely uniform, and it was possible that tadpoles reared in a tank that was polarized in respect to habitat types could come to orient themselves spatially using overhead cues alone. This interpretation makes sense since both rearing and test tanks were exposed to identical overhead cues (they were placed next to each other on the same bench). The right hand side of the test tank, in this way, corresponded to the right hand side of the rearing tank (high temperature, rock substrate). Therefore, while preferences of tadpoles had changed regarding substrate, they had learned and were using overhead cues to orient toward the "expected" location of the preferred,

Table 19. Summary of Rana initial choices in control tests and temperature and food entrainment groups. Left and Right designations indicate direction of choice from point of view of observer. One group was removed from Tank T at day 38 and later tested. A second group was left in Tank T at day 38 with the temperature gradient removed.

## Temperature Entrainment

Days Experience	Initial Left	Choices Right
1	$\frac{N}{12}$	$\frac{N}{8}$
4	$\frac{N}{17}$	$\frac{\text{Rock}}{3^\dagger}$
7	$\frac{N}{18}$	$\frac{\text{Rock}}{2^a}$
17	$\frac{N}{11}$	$\frac{N}{9}$
30	$\frac{N}{4}$	$\frac{\text{Rock}}{16^*}$
37	$\frac{\text{Rock}}{2}$	$\frac{N}{18^a}$
38	$\frac{N}{3}$	$\frac{N}{17^\dagger}$
Removed 2 Days	$\frac{N}{8}$	$\frac{N}{12}$
Removed 3 Days	$\frac{N}{6}$	$\frac{\text{Rock}}{14}$
Removed 9 Days	$\frac{N}{9}$	$\frac{N}{11}$
Removed 13 Days	$\frac{\text{Rock}}{3}$	$\frac{N}{17^a}$
Temp. off 2 Days	$\frac{N}{3}$	$\frac{N}{17^\dagger}$
Temp. off 8 Days	$\frac{N}{4}$	$\frac{N}{16^*}$

\* P &lt; .015

† P &lt; .003

a P &lt; .001

## Food Entrainment

Days Experience	Initial Left	Choices Right
7	$\frac{N}{22}$	$\frac{\text{Rock}}{3^a}$
30	$\frac{N}{9}$	$\frac{\text{Rock}}{16}$
37	$\frac{\text{Rock}}{10}$	$\frac{N}{15}$
38	$\frac{N}{4}$	$\frac{N}{21^a}$
54	$\frac{N}{6}$	$\frac{N}{19^*}$
57	$\frac{N}{16}$	$\frac{\text{Rock}}{4^*}$

## Control

4	$\frac{N}{17}$	$\frac{\text{Rock}}{3^\dagger}$
37	$\frac{\text{Rock}}{4}$	$\frac{N}{16^*}$
48	$\frac{N}{16}$	$\frac{\text{Rock}}{4^*}$
98	$\frac{N}{13}$	$\frac{\text{Rock}}{7}$

high temperature, habitat. Controls, reared in the absence of a temperature stimulus (Table 19), showed no preference was indicated. These tadpoles were exposed to the same overhead cues as experimental tadpoles.

Retention of learned behaviors was tested after isolating a group from tank T in a featureless tank (N) at day 38 with overhead cues different from the entrainment tank. Tests after 2, 3, 9, and 13 days of isolation showed that preferential responses using overhead cues and substrate were lost rapidly (by 2-3 days). But, complete loss of learned behavior was not evident until day 13 when tadpoles once again avoided substrate R.

A different effect was achieved by leaving a group in tank T at day 38 with the temperature gradient removed. Tests at 2 and 8 days demonstrated that responses to overhead cues were retained despite the absence of a temperature reinforcer. At least for this time period, overhead cues and/or substrates, if left unchanged, were sufficient reinforcers in themselves to maintain the learned behavior.

Experiment F was carried out simultaneously with the above series of experiments and similar results were obtained (Table 19). The first change in behavior was noted at day 30 (when group T reversed its preferences). Tadpoles showed no preference between substrates N and R (although more tadpoles selected R), nor when substrate positions were reversed on day 37. Tests on days 38 and 54 in a featureless test tank showed tadpoles could use overhead cues alone to orient toward the expected location of the high food habitat. But on day 57, when given a test of N:R, substrate R (right side) was avoided.



Although preferences of group F tadpoles were modified, a clear interpretation of these data is difficult. Assuming day 37 and day 38 tadpoles would behave alike, the data for these two days could mean that larvae were attending to both substrate and overhead cues and that because of this conflict on day 37 (rock was placed on the left side), neither side was preferred. When R was removed from the left side of the test tank on the following day, the "correct" side was chosen. But the results of day 57 confound this interpretation. The predicted outcome here would be a preference for the right side (R), or perhaps no preference if there existed a conflict between substrate preference and overhead cue preference. This result is inconsistent unless one infers that some change occurred late in the experimental regime itself. It is possible, for instance, that side N was not cleaned of food material frequently enough or that food over R was not offered often enough.

In conclusion, previous experiments using substrate alone showed tadpole responses to be highly stereotyped and unmodifiable by substrate rearing experience. The present experiments utilizing a more complex rearing regime demonstrate that substrate preferences are modifiable. Tadpoles can adapt should special situations arise and temperature and food are powerful stimuli to developing Rana tadpoles.

## DISCUSSION OF FACTORS THAT INFLUENCE TADPOLE DISTRIBUTIONS

## Oxygen

For an aquatic organism, oxygen may be an important limiting factor affecting distributions. Oxygen diffuses at a much slower rate through water than air. Solubilities of oxygen in water are affected by temperature, wind and wave action, and photosynthetic and respiratory activities of plant and animal life. For these reasons, oxygen levels vary appreciably with water depth and microhabitat characteristics in a body of water.

Tadpoles are capable of perceiving changes in oxygen tension, at least at low levels. As dissolved oxygen decreases below a critical level, tadpoles characteristically initiate "bobbing" behavior to and from the water surface (Wassersug and Seibert 1975; personal observations). Some tadpoles gulp surface air and utilize oral and pulmonary surfaces in addition to gills and skin for respiration. Water severely deficient in oxygen is avoided by larvae of R. temporaria (Costa 1967).

Field data in this study showed no correlation between oxygen levels and tadpole densities, (with the exception of Bufo in period II) when data were considered over the four daily time periods. Oxygen was never a significant variable in regression analyses. Hyla and Rana, whose distributions varied little in position throughout a daily cycle, experienced the high and low extremes in oxygen tension and exhibited no differential responses to such gradients. Bufo tadpoles also behaved randomly with respect to shifting oxygen gradients. The lowest readings

recorded in this study (commonly as low as 3.5 mg/l) were taken from within Bufo aggregations. This is due to the combined effect of relatively high water temperatures within aggregations and the respiratory activities of the tadpoles. Rana and occasionally Hyla were observed in the midst of these aggregations.

These observations alone, perhaps, are not sufficient to rule out oxygen as a potential factor influencing spatial distributions of these tadpoles but suggest that other factors are more important. There exist subtle variations in dissolved oxygen at the level of the microhabitat (e.g. between patches of vegetation) that could not be accurately accounted for in this study. But Rana and particularly Hyla regularly experienced "anoxic" conditions with no adverse effects. Bufo, on the other hand, seems more susceptible to long term anoxia (Wassersug and Seibert 1975; personal observations in lab). Unlike Rana and Hyla, bufonids probably do not gulp air when bobbing at the surface and do not develop functional lungs until metamorphosis (Savage 1951; Wassersug and Seibert 1975). Hence, bufonids may not have the physiological capacity to withstand prolonged exposure to anoxic water. If this is true, oxygen could be a factor involved in Bufo aggregating and schooling behavior. Although dense aggregations along a shoreline are commonly observed in the field through much of a day, these aggregations are temporary. Within a day, aggregations form, disperse, and reform elsewhere on a more or less continual basis. A limit of the ability to tolerate oxygen deficits within aggregations could be one factor constraining aggregation time in Bufo, thus influencing shifts in habitat selection behavior.

## Temperature

Temperature was highly correlated with distributions of Bufo, Hyla, and Rana at LTC. All species tended to select areas with the highest available temperatures. In regression models, temperature was the most significant variable related to Bufo densities and was a highly significant variable in all models selected for Hyla. The pronounced diel cycling in Bufo spatial distributions as observed in this study, and in another with B. americanus (Beiswenger 1977), reflects a tracking of changing thermal gradients by tadpoles. The relative importance of temperature as a variable was illustrated by the entrainment experiments with Rana. In that experiment, entrainment to the temperature cue was sufficient to modify stereotypic responses to substrate.

There are numerous reports of tadpoles of the genera Bufo, Rana, Hyla, Scaphiopus, and Pseudacris congregating in warmer areas of thermal gradients in the wild (Carpenter 1953; Mullally 1953; Brattstrom 1962, 1963; Karlstrom 1962; Brattstrom and Warren 1955; Tevis 1966; Bragg 1968; Ashby 1969; Beiswenger 1972). Whether tadpoles were actually responding to temperature or to some other factor was generally not clear in these studies. However, experimental evidence for selectivity in response to temperature by tadpoles has been recorded for several species (Workman and Fisher 1941; Beiswenger and Test 1967; Lucas and Reynolds 1967; Herreid and Kinney 1967; de Vlaming and Bury 1970; Beiswenger 1972). In these studies, tadpoles tended to select the highest temperatures available right up to physiological tolerable limits.

Temperature preferenda and critical thermal maxima and minima were not experimentally determined for the three species studied here. However, physiologically critical maximum and minimum temperatures are probably rarely experienced by tadpoles at LTC or at most other localities in the high Cascades. All species were recorded at the highest and lowest temperatures observed with no apparent ill effects. This is not to say that prolonged exposures to these extreme temperatures would not be detrimental. However, reported critical maximum temperatures for some tadpoles of 37 to 45°C (Karlstrom 1962; Heatwole et al. 1968; Wassersug 1973) are well above the highest temperatures recorded in this study.

The importance of temperature as a cue to tadpoles lies in its physiological influence on activity levels and general metabolism. Its most pronounced direct effect is on growth and developmental rates. Data for some temperate zone amphibians demonstrate an exponential decrease in developmental rate with temperature ranges from about 30 to 15°C and a linear decrease from 15 to about 4°C, where growth and development ceases (Atlas 1935; Ryan 1941; Moore 1942; Gosner and Black 1955). The importance of temperature as an ecological factor affecting growth, differentiation rate, size at metamorphosis, and timing of metamorphosis in larval amphibians has frequently been discussed in view of experimental findings (Kollros 1961; Etkin 1964; Wilbur and Collins 1973; Berven 1977; Berven et al. 1979; Smith-Gill and Berven 1979). Berven et al. (1979) argued that all of the variation they observed in life history characteristics of R. clamitans (length of the breeding season, time to metamorphosis, larval size at all stages, and rate of

larval development) could be attributed solely to the direct effect of temperature. For ectothermic organisms, temperature is correlated with activity and probably is also an important determinant of assimilation efficiencies of food matter, due to the temperature dependence of most biochemical processes.

All of these temperature-dependent factors can be considered important fitness components to a developing tadpole (Brockelman 1969; Wilbur 1972, 1977; Collins 1975; Smith-Gill and Gill 1978; Berven et al. 1979) and are probably effective in placing limits on the geographic range of a species. The three species studied here at high altitudes must complete larval development in a comparatively short period. If larvae fail to metamorphose prior to the onset of cold fall temperatures or habitat desiccation (situations that occur with some regularity in the Cascades), the results can be catastrophic. Finely-tuned responsiveness to temperature is one means by which tadpoles could effectively "self-regulate" (in this case maximize) growth and differentiation rates.

#### Depth

Depth was negatively correlated with species densities during both summer periods at LTC and figured significantly in some regression models. All species distributions were highly biased toward shallower depths close to shore at all Cascade pond and lake localities visited. Rana was never observed in water deeper than 45 cm and Hyla never deeper than 36 cm. Schooling and inactive Bufo frequently extended some distance from shore but were rarely observed in water deeper than 1 m,

and at these depths schools tended to be confined to the upper half of the water column.

There are anecdotal reports of tadpoles of various species being localized in shallow waters close to shore (Bragg 1940; Mullally 1953; Heyer 1973; Scott and Starret 1974; Brockelman 1969). However, to my knowledge, there is no experimental evidence to suggest that tadpoles can or cannot perceive changes in water depth or actually seek out habitats on the basis of water depth. The results of my experiments indicate that Rana and Bufo can sense differences in water depth or some factor correlated with water depth (e.g. light intensity, hydrostatic pressure).

In the laboratory, groups of Rana tadpoles were reared from early development at one of two depths (shallow or deep) and later preferred the depth at which they were reared. Eggs of Rana are laid in shallow water, often at the very limits of the shoreline. Subsequent to hatching, larvae remain attached to or in the immediate proximity of egg masses for 2-3 days. As tadpoles become active, they readily respond to temperature gradients and food (lab tests also show responses to substrates as early as 14 days of age). High temperatures and perhaps food (no direct evidence for the latter) are negatively correlated with depth. Even if depth preferences are not established early in larval development, other factors and responses to factors would tend to ensure that early stages develop in a shallow water environment. In this way, tadpoles could be conditioned through early experience to prefer shallow depths. But tadpoles that remain in the shallows at night (Rana and Hyla) do not take advantage of warmer temperatures present at this

time in deeper water. A possible benefit that might outweigh this apparent disadvantage is a reduced susceptibility to predation in the shallows at night. Depending on the pond or lake, these moderate depths are frequented at night by fish (Salmo) and salamander larvae (Ambystoma macrodactylum and A. gracile). The idea that tadpoles are especially vulnerable during inactive periods is consistent with the observation that these species seek refuge under objects at night. Bufo, in contrast, move to moderate depths at night (where water remains warmest) and do not seek refuge. Bufo are probably less susceptible to these predators due to their relative unpalatability and possible toxicity (Voris and Bacon 1966; Wassersug 1971; Cooke 1974; Heyer et al. 1975; Arnold and Wassersug 1978; Brodie et al. 1978). Trout were frequently observed to ignore passing schools of Bufo.

Wild Bufo were less selective in responses to depth in the laboratory. One group of wild Bufo preferred shallow water, avoided the moderate depth, and did not differ from random expectation in time spent in deepest water. In the field, Bufo are active over a wide range of depths, the deepest of which are considerably deeper than those tested. This latter condition could partly explain the lack of avoidance of the deepest depth tested. Responses in the field also are probably dependent on the activity mode and time of day. Dense aggregations form almost exclusively in very shallow depths and moving schools at moderate depths away from shore. Diel distributions generally reflect the tracking of highest available temperatures, but distribution shifts usually occur prior to warming or cooling of the



water. This suggests that Bufo initially respond to some other factor, possibly depth, and that these responses undergo photoperiodic shifts.

#### Shore Distance

Tadpole distributions associated with depth are correlated in the same way with distance from shore. Field design did not allow the separation of depth and shore distance as independent variables and shore distance was a variable difficult to test in the laboratory. The specific role of shore distance in habitat choice of tadpoles remains unclear.

The position of the shoreline is important to some orientation behaviors of larval anurans. "Y-axis orientation" (Ferguson and Landreth 1966; Ferguson 1967) involves the learning of shoreline cues and directing movements along an axis perpendicular to the shoreline (usually associated with escape responses). Y-axis orientation has been experimentally demonstrated in tadpoles of Bufo woodhousii and Rana catesbeiana (McKeown 1968; Goodyear and Altig 1971; Justis and Taylor 1976). Sun-compass and polarized light orientation (Auburn and Taylor 1979) have been implicated in the mechanism of the response. Many species of tadpoles, including those studied here, exhibit Y-axis responses in the field when disturbed or startled (personal observations). Indeed the eyes are positioned such that cues above the water surface could be readily perceived.

Multifactor entrainment experiments with Rana in the lab unexpectedly pointed out the importance of overhead cues in orientating responses. In this case, overhead cues were learned and attended to.

Shoreline cues (e.g. terrestrial or emergent vegetation, light intensity) could be used in conjunction with water depth in maintaining shoreline distributions and in confining activities to a particular section of shoreline in Rana and Hyla tadpoles.

Schooling Bufo could exploit these cues in maintaining their characteristic parallel course in respect to the shoreline. As Bufo become active in the early morning, initial orientations are directed toward the shore. Such movements frequently occur prior to any detectable change in temperature gradients. This suggests that tadpoles respond to shoreline cues or depth at this time.

#### Substrate

The physical and chemical qualities that make up a patch of substratum act largely to determine the composition of aquatic plant and animal colonizers (e.g. see Ruttner 1963; Minshall and Minshall 1977; Reice 1980). Substrate is one general characteristic that could be exploited by an organism as an indicator cue of suitable or unsuitable habitat.

Field observations linking substrate type with tadpole distribution are very rare in the literature. There is some experimental evidence, however, demonstrating that tadpoles selectively respond to features of a substrate. When tested in an artificial stream, Ascaphus truei tadpoles preferred substrates of smooth stones of a specific diameter (Altig and Brodie 1972). This was probably a tactile response related to the size of the oral sucking disc. Wiens (1970, 1972) used striped, square-patterned, and featureless substrates to condition and

subsequently test preferences of R. aurora and R. cascadae tadpoles. R. aurora reared in featureless or square-patterned habitats showed no preference for either striped or square-patterned substrates while stripe-reared larvae preferred the striped habitat. This preference, established within the first 2 weeks of conditioning, was retained after isolation from the substrate and could readily be renewed in young and old tadpoles. R. cascadae demonstrated selection only following conditioning in the square-patterned substrate. R. aurora tadpoles are often found in ponds containing linear images and shadows projected by the aquatic vegetation. The R. cascadae in Wiens' study were collected from a rocky-bottomed pond. Wiens hypothesized that tadpoles responded to images resembling the larval habitat. Stripes might resemble linear vegetation and squares a rocky substrate. Furthermore, tadpoles may respond selectively only after experience with the "optimal" substrate pattern (for that population or species) but not after experience with other "suboptimal" habitats. Tadpoles of eggs laid in suboptimal sites would therefore tend to disperse until the suitable habitat type was encountered. Quantitative field data in support of Wiens' hypothesis, however, were not available.

Punzo (1975) duplicated the methods and procedures of Wiens in his study of the Malayan painted frog, Kaloula pulchra. In this study, stripe-reared tadpoles preferred stripes, square-reared tadpoles preferred squares, and tadpoles reared in a featureless tray exhibited no preferences. Following a 1-week isolation period, stripe and square-reared tadpoles retained preferences for the familiar substrate. Punzo conjectured that a habitat selection mechanism based on learning is

adaptive in this species since females oviposit eggs in "optimal habitats". Young tadpoles would imprint on this habitat and tend to select that habitat type during development. However, no clear evidence was provided to support this hypothesis.

Contrasting stripes were also used by O'Hara (1974) to represent substrate types in an investigation of the ontogeny of choice behaviors in three species (R. sylvatica, B. americanus, and R. clamitans) of tadpoles. To emphasize midwater vs. bottom cues, stripes were affixed to the sides of an aquarium in one habitat, to the bottom in a second, and the third was lacking in the striped component (featureless). Choices of tadpoles were based on an avoidance of vertical stripes rather than a positive preference of one habitat over another. Although a habitat selection mechanism based on avoidance is an interesting concept in itself, it is difficult to impose a direct ecological interpretation of these data due to the artificial nature of the habitats. Unlike the species studied by Wiens and Punzo, choice behaviors of these species were not influenced by rearing regime.

Natural substrates were used in only one of the above studies (Altig and Brodie 1972). It is perhaps inappropriate than, to strictly interpret the remaining studies as evidence for the ontogeny of habitat selection behavior in tadpoles, as the ecological relevance of stripes or squares to a developing tadpole has not been determined. Rather, the usefulness of these studies is in pointing to the potentialities of choice behavior development. If one's purpose is to study the habitat selection process in an organism, the nature of the stimuli used is

critically important. Considerations must include the natural environmental context of early development.

Through the course of this field study, substrate was considered to be a major environmental variate associated with tadpole distributions. Tadpoles of Rana and Hyla in the field were rarely observed over sand and never observed over gravel or a predominately rock substrate. Observations include those at LTC and approximately 25 other Cascade lakes and ponds. Bufo displayed more generalized distributions with respect to substrate type in the field. Although there was a definite bias in frequencies toward finer substrates, tadpoles were observed over all available types, more so as the summer progressed (reflecting dispersal from sites of oviposition).

Laboratory tests with four substrates (smooth tank bottom, sand, gravel, and rock) showed that the finer grained substrates were consistently preferred by all species over coarser ones. Similar substrate pairs were not as clearly distinguished as were dissimilar pairings. Preferences were manifest early in development and persisted regardless of age, substrate experience, or population source. These findings for all species strongly corroborate field observations of tadpole substrate associations. Results are particularly significant because it was shown that wild tadpoles of varied backgrounds behave in much the same way as tadpoles reared in a relatively simple laboratory environment. Also, tadpoles from three different populations behaved alike. The sensory modalities used to distinguish substrates could be visual, tactile, or a combination of the two. Olfactory cues could be important in the field.

The findings for R. cascadae are contradictory to those of Wiens (1972). Tadpoles in his study were not only influenced by rearing experience, but selected a substrate pattern (squares) that Wiens hypothesized resembled a gravel or rock substrate. The source of this discrepancy could stem from the different substrate types used in our two experiments. Tadpoles may respond differently to an artificial substrate pattern as opposed to a natural stimulus. But because Wiens tested animals from a population that bred in a gravel-bottomed pond (no such population was sampled in the present study), his interpretation cannot be totally discounted. Individuals of populations living in dissimilar environments may respond differently to the same stimulus.

The present data and results from translocation experiments in the field imply that there is some common selective advantage associated with development over soft, fine substrates. When startled, Rana and Hyla tadpoles will sometimes hide just beneath the surface of soft substrates. This could be one mode of predator escape that could be utilized only over soft muddy substrates.

A critical variable directly associated with substrate was food. All three species feed on benthic particulates and the largest source of organics and chlorophyll was found in the muddy substrates. Hard, coarse substrates were relatively devoid of these. An adequate food supply is of course fundamental to maintaining some base level of growth. This is especially critical to larval anurans that develop in temporary or unpredictable environments. In these situations the timing of metamorphosis and the size at which larvae metamorphose may largely determine larval and juvenile survivorship (Wilbur 1972, 1977; Wilbur

and Collins 1973; Brockelman 1969; Collins 1975; Smith-Gill and Gill 1978; Smith-Gill and Berven 1979). Substrate could be utilized by tadpoles as a proximate indicator cue of relative food abundance.

### Vegetation

Two dominant macro-vegetation forms were present at LTC. Clumps of Carex were common at certain sites at the shore-water interface and often extended into the water as emergent clumps. Isoetes was prevalent as submergent vegetation. It was represented at every site locality and shore distance sampled but was most dense in the soft, muddy substrates in shallow to moderate depths. Neither species is ingested directly by tadpoles, but such vegetation may support a significant food source in the form of the attached aufwuchs. For example, Brockelman (1969) found a positive correlation between size at transformation and percent vegetation cover and a negative correlation of mean time at transformation with percent vegetation cover in B. americanus tadpoles reared in outdoor pens. In the present study, Isoetes was positively correlated with Bufo distributions and was a significant variable in some regression models. Carex was positively correlated with all species densities. It was a significant variable for Bufo and was a key variable for Hyla in that it explained more of the variation in Hyla density than any other variable or combination of variables.

Due to its widespread and near uniform distribution across shore distances, Isoetes would provide little usefulness as a key cue. All species were commonly observed over patches devoid of or densely populated with Isoetes. Carex was more restricted to certain sections

of gently sloping shorelines with soft substrates and in early summer served as an excellent predictor of where to find Rana, Hyla, and often Bufo. In addition to providing a surface area for the aufwuchs, the emergent clumps have the effect of reducing wave turbulence (wave disturbance visibly interferes with swimming and feeding activities of tadpoles) and providing potential areas of refuge from predators.

To test for the possible significance of Carex as a cue, clumps were simulated in the laboratory. When allowed to choose between vegetation and an open habitat, tadpoles of each species (lab-reared and wild) tended to prefer the open habitat. This suggests that although tadpoles were often found amongst vegetation, they may not be actively responding to the image of vegetation per se. Perhaps the results are equivocal due to the artificial nature of the stimulus. Although the general form of the stimulus approximated that conveyed by Carex, the reproduction was imperfect. It could also be conjectured that some cue in addition to a visual image (e.g. an odor) is necessary to elicit a response or to condition a response. Vegetation could be used as a supplemental or secondary cue, but the evidence suggests that it is not likely to be a primary variable influencing selection of a habitat. Indeed, Carex largely disappears as a cue from the aquatic habitat by midsummer even though tadpoles remain in the same general habitat types.

#### Social Factors

Aggregation behavior has been reported in few species of larval anurans. Among these are species of the genera Bufo, Scaphiopus, and Xenopus. These tadpoles form dense aggregates even in the apparent



absence of any patterning in external stimuli. Because individuals in each of these species seem to be responding to each other, their aggregations are classified as "social" (Bragg 1965; Beiswenger 1972; Wassersug 1973). Among species of other genera that have also been observed in dense aggregations are H. regilla (Brattstrom and Warren 1955; Brattstrom 1962), R. pretiosa (Carpenter 1953), R. boylei (Brattstrom 1962), and Rhinophrynus dorsalis (Stuart 1961). However, aggregations of these species are rare in nature and are possibly a result of responses to other stimuli (e.g. food or temperature) rather than conspecifics specifically.

Aggregative behavior of B. boreas is highly similar to that reported for B. americanus (Beiswenger 1972, 1975). During active periods, tadpoles spend most of their time in dense aggregations or in large, polarized schools. Hyla were almost never observed in "dense" aggregates in this study, but usually in loose groupings. An estimate of average nearest neighbor distance for Hyla (taken from measurements in 1976 at LTC and 1978 at Todd Lake; N = 97) in these loose groupings was 11 cm. The most common mode of association in Rana was also in loose groups. An estimate of nearest neighbor distance in this species (1976 at LTC and 1978 at Todd Lake; N = 84) was 13 cm. During the day, dense aggregates (tadpoles in contact with one another or within 2 cm distance) were occasionally observed in Rana (in less than 10% of the observations made on this species). These "clusters" were different in size and activity from Bufo aggregations. Numbers of tadpoles within clusters commonly ranged from four to approximately 80. Most numbered 10 to 30 in size. Unlike aggregating Bufo tadpoles, Rana were usually

inactive and non-feeding. If disturbed, clusters would commonly reform in 10 to 20 min.

Contagious distributions in nature could be nonsocial if tadpoles are simply responding to a common external stimulus or habitat type. A method of testing for visually mediated aggregation tendencies was developed by Wassersug and Hessler (1971). In that study, tadpoles of Xenopus laevis demonstrated mutual attraction toward conspecifics at early and late larval stages. Xenopus larvae are known to form natural aggregations. Two other species that are known to aggregate as tadpoles, Rhinophrynus dorsalis and B. boreas, exhibited nonrandom behavior in the test apparatus, but only the results for R. dorsalis clearly showed a tendency to aggregate. The five other species studied, Rana catesbeiana, R. pipiens, R. boylei, H. regilla, and Scaphiopus hammondi, showed no tendency to aggregate. Because there are incidental reports of these latter species forming natural aggregates, Wassersug suggested they are probably not a result of social mutual attraction but rather attraction to some common external factor.

Procedures similar to those utilized by Wassersug were employed in this study to test visually mediated aggregation tendencies in Rana, Hyla, and Bufo. Tests of wild and lab-reared tadpoles at varying developmental stages showed a strong aggregative tendency by all species. Results for Hyla were contrary to those obtained by Wassersug (1973) and results for Bufo were more clearly interpretable in my study. The differences between our studies could relate to differences in methodology or to the fact that Wassersug's experiments were not replicated (e.g. in one of four experiments run with Hyla in this study,

distributions showed no aggregation). Alternatively, different populations of the same species may demonstrate different aggregative tendencies. In any case, the findings reported here clearly implicate the presence of conspecifics as a factor affecting distributions of tadpoles. This was found even in species previously thought to lack conspicuous aggregation behavior (Rana and Hyla).

It is relevant to consider the possible functions of aggregation behavior because they may play a role in the habitat selection process. From field observations of H. regilla, R. boylii, B. boreas, and B. canorus, Brattstrom (1962) argued that temperature was the common factor that brings tadpoles together. Experimental evidence also suggested that compact groups increase water temperature at a faster rate than loose groups when both are exposed to an infrared lamp. Brattstrom suggested that tadpoles that continually select and experience the highest available temperatures could minimize developmental time and therefore escape the perils associated with a temporary aquatic habitat. Temperature has also been implicated in other studies (Brattstrom and Warren 1955; Beiswenger 1972, 1975) but Beiswenger concluded from his studies of B. americanus that temperature was only one possible factor and that the detection and efficient utilization of food was probably more directly related to aggregation behavior. Among other suggested functions are that aggregations aid in protecting tadpoles from leech infestation (Carpenter 1953; Bragg 1965) and that aggregations of "unpalatable", conspicuous species (i.e., Bufo) serve an aposematic function in deterring predators (Wassersug 1973). Wassersug also speculated that if Bufo groups consisted of related

individuals (siblings), kin selection might be operative. A preference to associate with familiar kin has since been demonstrated in B. americanus tadpoles (Waldman and Adler 1979).

Analyses of aggregation behavior in B. americanus by Beiswenger (1972, 1975, 1977) and Wilbur (1977a) can be applied to B. boreas behavior. Food procurement seems to be one important function of these aggregations. Shore aggregations effectively stir up loose organic materials from the substrate that tadpoles can then filter from the water. This may explain the absence of aggregations over hard, organically deficient substrates. Aggregations were never observed over such substrates at LTC and only observed on one occasion at another location (Todd Lake). Also, the temporary nature of aggregations may reflect transient depletion of food resources at that site. My data are not consistent, however, with Beiswenger's (1977) conclusion that natural aggregations do not create significant gradients in temperature. I have repetitive observations of temperature differentials of 2-3°C inside and outside aggregations across distances of only 10 to 20 cm. This increase in water temperature through solar heat absorption and perhaps metabolic activities could potentially play an important role in increasing developmental rates and reducing time to metamorphosis.

At least one factor, food enhancement, has been overlooked in published discussions of potential benefits accrued by individuals in aggregations. A large component of a tadpole diet consists of detrital and suspended particulates (Jenssen 1967; Kenny 1969; Wassersug 1975; Wassersug and Hoff 1979; Seale and Wassersug 1979). Detritus (non-

living) by itself is a low quality food source for most animals. It is rapidly passed through the gut and assimilation efficiency is generally low (Berrie 1975). Studies with some invertebrates and fishes indicate that the food quality of detrital material is directly related to the abundance of micro-organisms that colonize the detritus (Newell 1965; Darnell 1967; Berrie 1975; Kostalos and Seymour 1976; Bowen 1979; Cummins and Klug 1979). Micro-organisms themselves provide a food source of high quality (Burke 1933; Krogh 1931; Berrie 1975; Cummins and Klug 1979). Micro-organisms may be the only significant component of detritus that is actually assimilated by some aquatic invertebrates (Hargrave 1975) and tadpoles (Savage 1961; Wassersug 1975). Processing through the gut in turn augments recolonization of the fecal material by micro-organisms (Hargrave 1975). Aquatic organisms, including tadpoles, could in this way effectively enhance the quantity and quality of their food supply by re-ingesting conditioned fecal material. There is considerable evidence of this occurring in aquatic invertebrates (Hargrave 1975) and it is known that tadpoles allowed to ingest their feces realize enhanced growth over those that do not (Gromko et al. 1973; Steinwascher 1978a). Tadpoles may actually compete for feces when other particulate food sources are limited (Steinwascher 1978b). The benefit accrued through an enhanced food supply is thus a potentially important factor for tadpoles at low to moderate densities that remain within a delimited area without dispersing (e.g. Rana and Hyla) and those that move in large numbers in and out of areas (e.g. Bufo). Within limits, an increase in tadpole numbers facilitated by the tendency to aggregate may, in effect, increase rather than deplete a

local food supply. Indeed, the accumulation of tadpole fecal material (up to several mm thick) in the shallows of LTC and other sites was one obvious outcome of tadpole aggregations.

Some organisms can benefit by group living by reduced predation on group members (e.g. see discussions by Alexander 1974; Bertram 1978). Up to a point, a clumped prey population may be more difficult for a predator to detect (Taylor 1976, 1979), early predator detection by a group may be enhanced over that of an individual (Kenward 1978; Powell 1974; Siegfried and Underhill 1975), and an individual of a group may be less likely to be preyed upon (selfish cover seeking) (Hamilton 1971). These functions may be of importance in the Rana and Hyla aggregation mode. These species are more cryptic and probably more palatable as prey than Bufo tadpoles. Conspicuousness to predators in turn may place constraints on the size and density of Hyla and Rana aggregations compared with the Bufo mode. An intriguing prospect is the possible role that aggregating with kin could have in enhancing benefits (e.g. predator detection and avoidance, procurement of food) associated with group living (Sherman 1980). Tadpoles of R. cascadae (O'Hara and Blaustein, in press; Blaustein and O'Hara 1981) and B. americanus (Waldman and Adler 1979) discriminate between kin by preferring to associate with siblings over non-siblings in the laboratory. At present, however, there is no experimental evidence to suggest that clumping by tadpoles is an effective predator avoidance mechanism or that sibling groups actually occur in nature.

### Multiple Factors

Once it becomes known how organisms respond to single environmental factors, multiple factor experiments can be utilized to answer questions about possible hierarchies of cue preference, response adaptability given different variable combinations, and how variables interact in affecting choice responses. Although this kind of approach seems to be a promising means of unraveling complexities in the habitat selection process, few attempts have been made to test multiple variables in choice experiments (but see Sale 1968; Prince and Parsons 1977).

Rana tadpoles were reared in complex environments containing multiple cues. By associating a positive stimulus (high temperature or food) with a known negative stimulus (rock substrate), tadpole substrate preferences were modified. These findings, using natural cues, are significant because previous experiments had shown substrate preferences to be unaffected by substrate rearing regime. But even though tadpole distributions within the rearing tank became biased toward the rock substrate almost immediately, it took a relatively long period of time for behavioral modification to come about, and newly learned behaviors were lost rapidly when tadpoles were placed in a different habitat type. Perhaps if stimulus differentials were greater (i.e. higher temperature or more food), learning would be more rapid or be retained longer.

These experiments showed that there is the potential for behavioral adaptability regarding substrate preference in Rana and that in orientating behaviors temperature and food cues are attended to

preferentially over substrate cues. Tadpoles showed some ability to retain learned behaviors, but without some continual reinforcement these behaviors were lost. Also, tadpoles were capable of learning the spatial position of preferred habitats. Larvae selected the "expected" location of high temperature and high food habitats even in the absence of any temperature or food gradient. It might be advantageous to tadpoles to adapt behaviorally in certain fluctuating or changing environmental conditions (seasonal or yearly), if dispersal is costly, or if eggs are occasionally laid in suboptimal habitats. The observed entrainment to overhead cues suggests that these could also be important in orientating responses. Such cues could be used in maintaining shoreward distributions or in maintaining positions within the selected "home" area.



## SYNTHESIS

A striking outcome of this study is the apparent complexity of the habitat selection process in these tadpoles. Rather than there being one or two key cues (a frequent implicit assumption of many investigators), there appear to be several (Table 20). Tadpoles were responsive to cues of a structural, microclimatic, and biologic nature. Responses to certain cues were unaffected by rearing regime or developmental stage. Prior experience played a role in responses to one cue (depth). Stereotypic preferences to substrate were modified by conditioning in environments containing more than one cue, indicating that some adaptability cue preference is possible. Because experience may be important and because dispersal from suitable oviposition sites may be very low, oviposition site selection by the parents may itself be of some consequence to habitat selection behaviors that become manifest in their progeny.

Rana and Hyla tadpoles were similar in both habits and habitat associations. Each species was consistently recorded in high temperature habitats, in protected, gently sloping shallows close to shore, and over soft, muddy substrates. Distributions often coincided with patches of vegetation (Carex, Isoetes), but this was not a consistent trend at the microhabitat level. Hyla tended to be more widely dispersed across sites, but neither species dispersed significantly from oviposition areas. Both species remained in the shallows at night and sought cover underneath logs or other objects when these were available. During the day, both species were most commonly

Table 20. Summary of tadpole habitat preferences. Descriptions with upper case letters denote a stronger relative response than lower case descriptions.

Variable	<u>Rana cascadae</u>		<u>Hyla regilla</u>		<u>Bufo boreas</u>	
	Field	Lab	Field	Lab	Field	Lab
Temperature	HIGH OVER LOW (DAY)	HIGH OVER LOW	HIGH OVER LOW (DAY)	HIGH OVER LOW	High over Low (Day and Night)	HIGH OVER LOW
Substrate	FINE OVER COARSE	FINE OVER COARSE	FINE OVER COARSE	FINE OVER COARSE	Fine over Coarse	FINE OVER COARSE
Food	YES	YES	YES	YES	YES	YES
Social Factors	GREGARIOUS	GREGARIOUS	GREGARIOUS	GREGARIOUS	GREGARIOUS	GREGARIOUS
Depth	SHALLOW	DEPENDENT ON EXPERIENCE	SHALLOW	?	Shallow (Time of Day)	Shallow
Vegetation	VEGETATION	OPEN WATER	VEGETATION	OPEN WATER	Vegetation	OPEN WATER
Shore Distance	CLOSE TO SHORE	?	CLOSE TO SHORE	?	Close to Shore	?
Dissolved Oxygen	NO	?	NO	?	NO	?

found in loose, patchily distributed groupings of conspecifics, but Rana were also occasionally observed in small but dense aggregations.

Although Bufo frequented the same habitat types as Rana and Hyla, their habits and activities differed considerably. Bufo tadpoles underwent diel shifts in distributions from moderate depths at night when they were inactive and randomly spaced on the bottom to predominately shallow distributions during the day. These shifts are probably triggered by light intensity or photoperiod (Beiswenger 1977; personal observations) and probably function in placing tadpoles in the warmest areas of the lake through a daily cycle. Unlike Rana and Hyla, Bufo tadpoles were far more numerous, dispersed widely around a lake shore, and were found in every habitat type available at LTC except deep water areas. During their active period, Bufo occur in dense, stationary aggregations along the shoreline or in large polarized schools that travel parallel to the shore. Bufo exploit suspended particulates in the water column for food in addition to substrate-bound food.

Field observations and marking experiments indicated that Rana and Hyla are low dispersers at LTC and other Cascade localities. They tended to remain in the vicinity of their oviposition site throughout larval development and hence experienced just one generalized habitat type during larval life. It could be argued that these species are habitually sedentary and simply remain in the vicinity of the oviposition site without actively selecting a particular habitat. Evidence from larval translocations, however, suggests that the tadpoles are selective. They tended to remain in habitats similar to those in

which they were collected and dispersed from nonpreferred types. Dispersal could be important in young larvae if eggs were laid in a nonpreferred habitat type, if density levels exceeded the carrying capacity of the occupied habitat, or if seasonal changes (e.g., drops in water level) make the habitat unsuitable.

In the study of any behavioral pattern, one hopes ultimately to gain some understanding of the selective constraints that have operated to produce the observed behavior and thereby attribute some adaptive significance to the behavior. For habitat selection behavior, this becomes a problem of establishing links between habitat quality and individual fitness. To accomplish this, it would be necessary to compare fitness in preferred and less preferred habitats and show that in chosen habitats the animal has the greatest probability of survival and reproductive success. The lack of this kind of information attests to the difficulty of achieving such a level of resolution for most organisms. However, habitat selection behavior associated with differentials in fitness has been reported for aphids (Whitham 1980), and habitat preferences correlated with genetic polymorphisms have been noted for several other species (Jones 1980). Theoretically, a knowledge of the array of environmental cues to which organisms are most responsive can yield insights into the kinds of factors that have historically been important. Behavioral responses to real environmental cues or situations should be under the influence of natural selection. As Wiens (1976) noted in this context, organisms "should be attuned to the properties that produce fitness differentials". This does not mean,

however, that there is always a direct relationship between the tactics an organism uses to select a habitat and the strategy of the behavior.

Habitat selection could function in reducing predation. Known predators of the tadpoles I studied are aquatic insect larvae (odonates, hemipterans, coleopterans [dytiscids and hydrophilids]), salamander larvae (Ambystoma), snakes (Thamnophis), and birds (robins, gray jays, shorebirds). Potential predators at some localities are leeches, fish (Salvelinus, Salmo), and perhaps some mammals (e.g. raccoons). In the Cascades, the primary tadpole predators are insect and salamander larvae. If present in sufficient numbers, insect larvae are capable of severely reducing tadpole populations (Herreid and Kinney 1966; Heyer et al. 1975; Heyer and Muedeking 1976; Caldwell et al. 1980) as are salamander larvae (Calef 1973). Year old Ambystoma macrodactylum larvae are capable of totally eliminating populations of Hyla and Rana from a pond (personal observations over a 4-yr period at a single pond). Although incidental observations suggesting habitat partitioning between tadpoles and aquatic insect predators have been reported in other anuran species (Heyer et al. 1975; Heyer and Muedeking 1976; Caldwell et al. 1980), this does not seem to be the case for the species studied here. Highest concentrations of insect predators tended to coincide in habitat distribution with tadpoles. Salamander larvae also frequent these shallow areas, especially at night. Because tadpole distributions are in the extreme shallows along shorelines, vulnerability to snake, bird, and mammal predators is also high. Although Bufo are apparently unpalatable to some predators (Wassersug 1971; Heyer et al. 1975; Brodie et al. 1978), some birds, snakes, and predaceous insect larvae

will prey on them (Wassersug 1973; Brodie et al. 1978; personal observations).

The presence and abundance of any of these predators can vary greatly between breeding sites in the Cascades. For example, at LTC there were no snakes or salamander larvae present and the abundance of insect larval predators was low. However, other localities were visited where either snakes, salamanders, or insects were high in abundance. If predation is a frequent and powerful selective agent influencing tadpole habitat selection behavior, one would expect tadpole distributions to reflect this pressure in populations exposed to it. But as previously noted, tadpoles were found in remarkably similar habitat types regardless which predators were locally abundant. Although predation is undoubtedly an important source of tadpole mortality in some breeding habitats, it is probably not the major selective force influencing habitat selection behavior in these populations.

Both intra- and interspecific competitive interactions among tadpoles may be important under certain conditions and could lead to habitat partitioning. The phenomenon of chemical growth inhibition (e.g. Rose 1960; Richards 1962; Akin 1966; Licht 1967) is one type of interference competition observed in some crowded tadpole populations. Diminished growth, increased larval period length, and unequal size distributions have been associated with increased tadpole densities in several species (Brockelman 1969; DeBenedictis 1974; Wilbur and Collins 1973; Wilbur 1976, 1977a, 1977b; Steinwascher 1979; Collins 1979). Exploitation competition for food has been implicated in other studies (DeBenedictis 1974; Wassersug 1975; Wilbur 1977a; Steinwascher 1978a,

1979). Suggestions of interspecific competitive interactions (Dumas 1964; DeBenedictis 1974; Wiltshire and Bull 1977) and possible habitat partitioning between tadpole species (Heyer 1973, 1974) have also been reported.

In this study, there was no clear habitat segregation between Rana, Hyla, and Bufo. All species selected similar habitat types and were often found in the same habitat. Because of this overlap and the tendency for tadpoles to aggregate, the potential for competition would seem to be great. No direct evidence of competition was obtained in this study. Displacement of one species due to activities of another, however, was occasionally observed between two of the species. The formation of dense Bufo aggregations in an area where Hyla were present would sometimes result in a displacement of Hyla to the outer edge of the Bufo aggregation.

The fact that the different species frequently overlap in distribution suggests that intense interspecific competition may be infrequent in these populations. But a pertinent consideration is that suitable tadpole habitats in lakes themselves are limited in availability. Successful metamorphosis outside preferred habitats may be close to zero. In these circumstances, clear habitat segregation between species, except in terms of spatial segregation, should perhaps not be expected. Rather, one might expect to see changing patterns of species presence and absence (or spatial segregation) depending on the given ecological setting and the competitive superiority of a species over others in that situation.

Tadpoles have been depicted as being a life history stage specialized for feeding and growing (Wassersug 1975). Indeed, breeding by anurans usually coincides with a time when larvae can make maximal use of temporary increases in primary production. Factors that affect growth and developmental patterns are probably major determinants of larval "fitness" in anurans. It is generally conceded that recent growth history is a strong determinant of metamorphic and juvenile success (Brockelman 1969; Wilbur 1972, 1976, 1977a; Wilbur and Collins 1973; DeBenedictis 1974; Smith-Gill and Gill 1978; Smith-Gill and Berven 1979; Steinwascher 1979; Travis 1980). Rapid growth and large size have been associated with superior intra- and interspecific competitive ability in larval anurans (Wilbur and Collins 1973; Collins 1975; Wassersug 1975; Wilbur 1977a; Wiltshire and Bull 1977; Steinwascher 1978a, 1978b, 1979; Smith-Gill and Gill 1978; Travis 1980). Larger size at transformation can increase post-metamorphic survival (Shvarts and Pyastolova 1970; DeBenedictis 1974), result in less stress due to desiccation (Claussen 1969), increase juvenile overwintering survival (Martof 1956), and may result in a lowering of the age of first reproduction (Martof 1956; Salthe and Duellman 1973; Collins 1975). Larger adult size increases mating success in some anurans (Wells 1977; Wilbur et al. 1978; Howard 1979a, 1979b, 1980), and has been associated with larger clutch and egg sizes of females (Salthe and Duellman 1973; Salthe and Mecham 1974; Collins 1975; Kuramoto 1978; Howard 1979b) and multiple ovipositions per year (Howard 1979a). Tadpoles that grow rapidly can also achieve a size refuge from predators at an earlier age



(DeBenedictis 1974; Heyer et al. 1975; Heyer and Muedeking 1976; Caldwell et al. 1980).

The length of time that it takes to reach the metamorphic transformation period is especially crucial to anurans that breed in ephemeral ponds or sharply seasonal environments (e.g. breeding localities in the Cascades). Selection in these environments should favor rapid growth. At higher elevations it takes longer for snow and ice to melt from breeding areas, temperatures are cooler, and the onset of winter occurs sooner than at lower altitudes. The length of life of temporary ponds is primarily governed by quantities of winter and summer precipitation, which may fluctuate widely from year to year. For example, precipitation levels were well below normal in 1977 in Oregon and resulted in premature evaporation of ponds by as much as 4-6 weeks. Few of the temporary breeding areas lasted long enough for larvae to reach metamorphosis. In one case, a population extinction (R. cascadae at LTC) coincided with the drought. But even in more "normal" years, it was not uncommon to observe breeding sites that dried before all tadpoles were able to transform. The onset of cold and sub-zero temperatures in the fall is also unpredictable and can severely affect pre-metamorphic and newly transformed frogs. Newly transformed individuals are particularly vulnerable to desiccation and sub-zero temperatures. I witnessed on one occasion at LTC (1976) what appeared to be a large proportion of the Bufo larval population that had frozen in the shallows the prior year before they were able to metamorphose. My observations indicate that this source of mortality is frequent (although usually not as catastrophic) for at least some larvae at some

localities nearly every year. Young juveniles are also strongly dependent on an abundant terrestrial food supply (primarily insects) which may no longer be available if metamorphosis has been prolonged. The advantage of fast growth in ephemeral environments is clearly evident in those species possessing distinct fast and slow growing morphs (Bull 1977; Merrel 1972). In years when the growing season is short (i.e. dry and hot), fast growing morphs are far more successful than slow growers.

Whatever the major environmental component — predation, competition, or habitat ephemerality — larval growth and development rates are probably major determinants of metamorphic, early juvenile, and possibly adult success. Traits that affect growth are likely to be closely linked to overall fitness and are probably strongly influenced by natural selection.

Tadpole habitat selection behaviors reflect intense selection for rapid growth. Preferred substrate types are associated with areas of high food availability. Shallow depths and shoreward distributions are related to high temperatures and probably higher relative food supplies. All species are strongly responsive to temperature and will respond to this factor preferentially over other cues. Temperature is a powerful regulator of metabolism and growth in ectotherms and may facilitate digestive and food assimilation efficiencies. By aggregating with conspecifics, tadpoles might be able to seek out and better exploit a food resource, increase the surrounding water temperature (i.e. Bufo), and possibly cultivate and enhance the nutritive quality of their food supply. Adults also play an important role by placing tadpoles in an

optimal environment for early growth. Eggs are laid in shallow protected areas where solar heat absorption is near maximum and where the water reaches the highest temperature. All species are explosive breeders in the high Cascades and often lay eggs immediately after the ice recedes from pond and lake margins. This, in effect, maximizes the time available for tadpoles to grow and utilize the aquatic environment. Tadpoles that undergo development in less temporal environments than those in the Cascades (e.g., some lower elevation populations in the Willamette Valley) might be expected to show less habitat selectivity or preferences more strongly correlated with predator or competitor distributions. The pronounced habitat selection behaviors of the tadpoles and adults studied in this investigation, however, reflect an overall strategy to minimize the time needed for successful completion of metamorphosis by maximizing components of growth and development.

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## APPENDICES



## APPENDIX I

In these tables the raw data (means) are presented for each variable in early and late summer periods in 1976. For each summer period, tables are categorized in respect to time of day (four time periods) data were collected. A complete data table is given for one time period only (0600 - 0900) in each summer period because variable means other than species densities, temperature, and oxygen remained the same. Data were collected at three distances from shore (ShD's of 0.3, 3.5, and 8.0 m) at each lake perimeter site (designated by indicator variables 0 and 1). An average of over 400 observations were taken at each perimeter site, each summer period.

Early Summer

0600 - 0900

No./56m <sup>2</sup>			Perimeter Site											Substrate % Coverage				Vegetation Index						
Bufo	Hyla	Rana	A	B	C	D	E	F	G	H	I	J	ShD	Temp	O <sub>2</sub>	Depth	Mud	Sand	Grav.	Rock	Open	Isoetes	Carex	Log
3.4	1.9	1.8	1	0	0	0	0	0	0	0	0	0	0.3	13.2	5.95	7.5	73	11	10	6	2	3	3	5
7.8	0.1	1.4	1	0	0	0	0	0	0	0	0	0	3.5	14.5	6.91	36.0	91	5	0	4	1	4	1	1
10.6	0	0	1	0	0	0	0	0	0	0	0	0	8.0	15.0	7.12	63.0	99	0	0	1	0	3	0	0
2.3	0.3	0	0	1	0	0	0	0	0	0	0	0	0.3	12.5	6.38	11.5	12	26	62	0	4	1	1	3
4.8	0	0	0	1	0	0	0	0	0	0	0	0	3.5	14.1	7.08	48.0	20	35	45	0	2	2	0	2
12.9	0	0	0	1	0	0	0	0	0	0	0	0	8.0	14.6	6.95	81.0	100	0	0	0	0	3	0	0
4.8	4.3	0	0	0	1	0	0	0	0	0	0	0	0.3	13.7	6.26	5.5	95	5	0	0	1	4	5	2
15.2	0.4	0	0	0	1	0	0	0	0	0	0	0	3.5	14.4	7.02	24.0	100	0	0	0	1	5	2	1
16.7	0	0	0	0	1	0	0	0	0	0	0	0	8.0	15.2	7.05	66.5	100	0	0	0	0	5	0	0
2.7	2.1	6.0	0	0	0	1	0	0	0	0	0	0	0.3	12.5	6.61	9.0	70	17	13	0	3	2	5	4
14.6	0.8	4.7	0	0	0	1	0	0	0	0	0	0	3.5	13.9	7.26	39.0	88	8	4	0	2	3	2	3
20.9	0	0	0	0	0	1	0	0	0	0	0	0	8.0	14.5	7.08	75.0	100	0	0	0	1	4	0	0
3.3	2.1	0	0	0	0	0	1	0	0	0	0	0	0.3	13.2	6.40	6.5	84	10	6	0	2	4	4	2
8.0	1.1	0	0	0	0	0	1	0	0	0	0	0	3.5	14.0	7.16	22.0	100	0	0	0	1	5	1	1
16.8	0	0	0	0	0	0	1	0	0	0	0	0	8.0	15.2	6.93	52.5	100	0	0	0	0	4	0	2
0.5	0	0	0	0	0	0	0	1	0	0	0	0	0.2	13.2	7.07	13.0	12	23	35	30	4	1	1	0
3.4	0	0	0	0	0	0	0	1	0	0	0	0	1.0	14.3	6.92	100.0	100	0	0	0	2	2	0	0
1.1	2.2	0	0	0	0	0	0	0	1	0	0	0	0.3	13.0	7.12	8.0	68	24	8	0	3	3	4	1
6.8	0	0	0	0	0	0	0	0	1	0	0	0	3.5	13.9	7.38	31.5	91	9	0	0	2	4	1	1
7.9	0	0	0	0	0	0	0	0	1	0	0	0	8.0	14.5	7.06	68.0	100	0	0	0	1	4	0	0
0.9	0.9	0	0	0	0	0	0	0	0	1	0	0	0.3	13.8	6.51	10.0	63	18	12	7	3	2	2	3
9.0	0.1	0	0	0	0	0	0	0	0	1	0	0	3.5	15.0	6.74	42.0	94	6	0	0	2	3	0	2
8.9	0	0	0	0	0	0	0	0	0	1	0	0	8.0	15.3	6.85	85.5	100	0	0	0	1	4	0	1
0.3	0	0	0	0	0	0	0	0	0	0	1	0	0.2	11.8	7.24	16.5	13	9	15	63	4	5	0	1
1.4	0	0	0	0	0	0	0	0	0	0	1	0	1.0	13.0	7.10	117.0	100	0	0	0	2	2	0	1
2.1	0.3	0	0	0	0	0	0	0	0	0	0	1	0.3	11.8	6.60	12.0	41	31	23	5	4	1	2	2
8.6	0	0	0	0	0	0	0	0	0	0	0	1	3.5	13.2	7.21	45.5	62	22	14	2	3	2	0	1
5.9	0	0	0	0	0	0	0	0	0	0	0	1	8.0	14.2	7.06	94.0	94	5	0	1	2	3	0	1

## 0900 - 1200

<u>Bufo</u>	<u>Hyla</u>	<u>Rana</u>	<u>Temp</u>	<u>O<sub>2</sub></u>
11.6	4.5	4.3	16.6	8.73
18.3	0.5	2.0	15.6	8.35
10.9	0	0	15.5	8.06
6.1	0.8	0	15.4	8.55
18.0	0.1	0	14.3	8.53
9.4	0	0	14.0	8.07
24.3	6.7	0	17.0	8.96
31.0	0.8	0	15.1	8.78
16.4	0	0	15.0	8.21
12.7	3.4	10.6	16.8	8.70
21.7	1.0	4.7	15.2	8.83
13.2	0	0	14.2	8.10
13.8	5.8	0	18.0	8.35
26.9	0.7	0	16.1	8.19
15.7	0	0	15.1	8.30
2.9	0	0	14.6	8.63
10.0	0	0	14.6	8.49
11.6	2.8	0	16.6	8.29
16.0	0	0	14.3	8.77
11.6	0	0	14.2	8.06
10.1	1.2	0	15.9	8.87
22.4	0	0	15.0	8.60
8.5	0	0	14.8	7.98
0.6	0	0	14.8	8.46
4.3	0	0	13.5	8.27
5.1	0.5	0	15.2	8.47
11.9	0	0	14.5	8.42
4.9	0	0	14.3	7.80

## 1200 - 1500

<u>Bufo</u>	<u>Hyla</u>	<u>Rana</u>	<u>Temp</u>	<u>O<sub>2</sub></u>
56.6	6.6	6.9	22.4	8.60
30.2	0.7	4.0	17.6	9.82
7.5	0	0	16.8	9.76
11.7	1.3	0	20.0	8.91
23.9	0	0	15.5	9.61
3.7	0	0	14.6	10.16
82.8	10.6	0	23.5	8.64
41.5	2.7	0	18.3	9.67
12.7	0	0	16.5	9.80
44.9	8.1	11.8	23.0	8.55
25.6	0.8	3.9	18.5	9.77
7.6	0	0	15.2	9.98
87.3	10.6	0	23.5	8.34
34.5	0.9	0	20.1	9.26
11.9	0	0	17.2	9.70
1.9	0	0	19.2	8.90
7.4	0	0	16.3	9.69
35.1	4.6	0	21.6	8.68
10.4	0	0	17.6	9.79
5.1	0	0	15.7	9.83
33.5	2.2	0	21.8	8.68
22.5	0	0	17.3	9.76
6.9	0	0	15.5	9.99
1.1	0	0	19.3	8.88
6.4	0	0	14.3	9.68
14.8	0.7	0	19.9	8.57
14.9	0	0	16.0	9.93
3.2	0	0	15.0	9.80

## 1500 - 1800

<u>Bufo</u>	<u>Hyla</u>	<u>Rana</u>	<u>Temp</u>	<u>O<sub>2</sub></u>
68.6	6.9	6.6	24.2	8.27
32.8	0.7	2.9	20.2	8.64
4.5	0	0	18.1	9.13
31.1	0.9	0	23.1	8.30
26.0	0	0	18.8	8.95
7.5	0	0	15.7	9.53
94.8	10.4	0	25.0	8.25
38.4	2.8	0	21.5	8.70
13.2	0	0	18.6	9.00
51.1	8.6	11.8	25.2	8.29
29.5	0.6	4.9	21.6	8.65
9.0	0	0	16.9	9.33
83.6	7.8	0	25.3	8.16
38.9	1.7	0	22.4	8.55
14.2	0	0	20.1	8.73
3.7	0	0	23.5	8.38
10.7	0	0	19.5	8.72
31.1	5.0	0	23.9	8.26
9.4	0	0	21.0	8.52
3.9	0	0	18.8	8.69
35.9	3.1	0	23.1	8.44
25.9	0	0	20.1	8.72
6.5	0	0	16.9	9.11
1.4	0	0	22.5	8.37
8.1	0	0	15.4	9.06
24.3	1.0	0	23.3	8.42
17.9	0	0	19.0	8.81
2.4	0	0	15.8	9.31

Late Summer

0600 - 0900

No./0.56m <sup>2</sup>			Perimeter Site										ShD	Temp	O <sub>2</sub>	Depth	Substrate % Coverage				Vegetation Index			
Bufo	Hyla	Rana	A	B	C	D	E	F	G	H	I	J					Mud	Sand	Grav.	Rock	Open	Isoetes	Carex	Log
4.6	3.8	4.1	1	0	0	0	0	0	0	0	0	0	0.3	14.8	5.45	11.0	91	5	0	4	1	4	1	1
10.9	3.4	2.6	1	0	0	0	0	0	0	0	0	0	3.5	16.0	6.50	41.5	99	0	0	1	0	5	0	0
13.2	0	0	1	0	0	0	0	0	0	0	0	0	8.0	16.2	6.86	59.5	99	0	0	1	0	5	0	0
3.0	1.4	0	0	1	0	0	0	0	0	0	0	0	0.3	15.1	5.93	13.0	55	15	30	0	2	1	0	2
7.8	1.9	0	0	1	0	0	0	0	0	0	0	0	3.5	15.8	6.72	45.0	100	0	0	0	0	4	0	1
18.5	0	0	0	1	0	0	0	0	0	0	0	0	8.0	15.9	6.70	72.0	100	0	0	0	0	5	0	0
4.8	7.2	0	0	0	1	0	0	0	0	0	0	0	0.3	15.0	5.97	9.5	100	0	0	0	0	4	2	1
17.1	8.3	0	0	0	1	0	0	0	0	0	0	0	3.5	15.4	6.79	21.0	100	0	0	0	0	5	0	1
22.3	0	0	0	0	1	0	0	0	0	0	0	0	8.0	16.0	7.08	57.5	100	0	0	0	0	5	0	0
3.2	3.3	7.8	0	0	0	1	0	0	0	0	0	0	0.3	14.5	6.11	7.5	83	12	5	0	1	3	2	3
18.4	2.8	4.2	0	0	0	1	0	0	0	0	0	0	3.5	15.6	6.73	37.0	100	0	0	0	1	4	0	1
20.7	0	0	0	0	0	1	0	0	0	0	0	0	8.0	15.6	6.90	78.0	100	0	0	0	0	4	0	0
2.1	5.8	0	0	0	0	0	1	0	0	0	0	0	0.3	13.9	6.46	7.0	96	4	0	0	1	4	2	1
9.8	3.0	0	0	0	0	0	1	0	0	0	0	0	3.5	14.8	6.75	18.5	100	0	0	0	0	5	0	1
28.3	0	0	0	0	0	0	1	0	0	0	0	0	8.0	15.6	7.07	63.0	100	0	0	0	0	4	0	2
1.0	0	0	0	0	0	0	0	1	0	0	0	0	0.2	14.8	6.20	15.5	86	8	6	0	2	2	1	0
4.4	0	0	0	0	0	0	0	1	0	0	0	0	1.0	15.8	7.12	89.5	100	0	0	0	1	3	0	0
4.2	2.9	0	0	0	0	0	0	0	1	0	0	0	0.3	14.4	6.33	8.0	92	8	0	0	1	3	2	1
9.0	0.8	0	0	0	0	0	0	0	1	0	0	0	3.5	15.3	6.68	29.5	100	0	0	0	0	4	0	0
10.1	0	0	0	0	0	0	0	0	1	0	0	0	8.0	15.5	7.00	61.0	100	0	0	0	0	4	0	0
5.5	0.7	0	0	0	0	0	0	0	0	1	0	0	0.3	14.6	6.29	12.0	93	7	0	0	2	2	1	3
11.5	0	0	0	0	0	0	0	0	0	1	0	0	3.5	15.7	6.77	34.5	100	0	0	0	0	3	0	2
15.4	0	0	0	0	0	0	0	0	0	1	0	0	8.0	15.7	7.01	75.0	100	0	0	0	0	3	0	1
0.8	0	0	0	0	0	0	0	0	0	0	1	0	0.2	15.4	6.40	15.5	60	0	6	34	3	1	0	0
3.6	0	0	0	0	0	0	0	0	0	0	1	0	1.0	15.9	7.24	108.0	100	0	0	0	1	2	0	0
2.3	0.1	0	0	0	0	0	0	0	0	0	0	1	0.3	14.9	6.24	10.0	63	18	16	3	2	2	1	2
7.9	0	0	0	0	0	0	0	0	0	0	0	1	3.5	15.5	6.67	42.0	82	11	5	2	2	2	0	1
9.4	0	0	0	0	0	0	0	0	0	0	0	1	8.0	15.8	7.18	85.5	100	0	0	0	1	2	0	1

## 0900 - 1200

Bufo	Hyla	Rana	Temp	O <sub>2</sub>
12.0	6.5	5.6	18.5	8.66
23.5	1.7	1.1	17.4	8.52
12.3	0	0	16.7	8.10
7.3	4.0	0	17.9	8.71
18.6	1.8	0	16.6	8.68
15.9	0	0	16.5	8.14
21.2	9.3	0	19.9	8.45
33.4	2.1	0	16.7	8.64
29.0	0	0	16.5	8.23
11.1	6.8	8.8	19.4	8.40
24.7	1.0	4.5	15.9	8.58
20.6	0	0	15.9	8.49
25.0	7.4	0	19.7	8.52
28.7	0.6	0	17.5	8.90
15.5	0	0	16.4	8.47
6.0	0	0	17.0	8.61
13.4	0	0	16.0	8.48
8.9	4.2	0	18.1	8.27
23.6	0.8	0	17.3	8.70
17.4	0	0	16.1	8.81
13.8	2.6	0	17.8	8.20
24.1	0	0	16.7	8.53
16.5	0	0	15.9	8.50
1.0	0	0	16.5	8.22
6.9	0	0	16.1	8.65
8.3	0	0	16.9	8.34
12.1	0	0	16.0	8.41
8.5	0	0	16.0	8.40

## 1200 - 1500

Bufo	Hyla	Rana	Temp	O <sub>2</sub>
44.6	7.6	5.3	24.5	8.03
31.4	1.1	2.0	20.0	8.65
10.0	0	0	19.8	8.71
14.3	6.0	0	23.3	8.16
29.2	1.9	0	19.0	8.88
12.5	0	0	18.8	9.04
74.9	12.4	0	25.4	7.96
44.7	3.0	0	19.9	8.60
15.2	0	0	19.2	8.79
35.8	6.2	10.1	24.1	7.91
28.6	2.1	2.9	18.9	8.80
11.7	0	0	18.7	8.91
81.2	8.7	0	25.8	8.77
40.7	1.2	0	20.9	8.52
17.9	0	0	19.4	8.78
19.0	0	0	22.2	8.09
22.5	0	0	18.5	8.79
36.9	8.1	0	23.8	7.96
21.4	0.6	0	19.9	8.61
15.0	0	0	18.6	8.89
42.3	3.0	0	23.8	7.91
23.4	0	0	18.7	8.87
9.6	0	0	18.5	9.00
3.8	0	0	22.0	8.10
5.1	0	0	18.0	8.82
13.0	0.2	0	23.0	8.01
8.0	0	0	18.6	8.63
5.9	0	0	18.2	8.68

## 1500 - 1800

Bufo	Hyla	Rana	Temp	O <sub>2</sub>
40.2	7.0	6.8	25.6	7.64
28.9	2.0	1.2	21.0	8.00
8.7	0	0	21.0	7.95
27.4	6.5	0	24.4	7.61
21.2	2.3	0	19.6	8.37
13.7	0	0	19.4	8.42
70.3	13.9	0	25.9	7.56
37.1	2.7	0	20.7	8.19
17.3	0	0	20.4	8.30
41.2	5.6	9.3	25.0	7.62
25.7	2.0	2.9	19.8	8.41
13.5	0	0	19.5	8.40
70.4	7.9	0	26.1	7.59
44.9	1.6	0	22.0	8.17
20.1	0	0	20.4	8.28
23.3	0	0	23.1	7.73
20.8	0	0	19.1	8.46
41.2	6.8	0	24.6	7.82
17.5	1.4	0	20.6	8.40
16.5	0	0	19.6	8.36
38.6	3.2	0	24.4	7.75
27.3	0.1	0	19.8	8.53
14.1	0	0	19.4	8.50
5.0	0	0	22.7	7.69
9.9	0	0	18.8	8.27
16.4	0.3	0	23.9	7.68
9.2	0	0	19.6	8.41
4.6	0	0	19.2	8.36

## APPENDIX II

Product-moment correlation matrices are given for early and late summer periods at LTC. All variables are included except the 10 perimeter sampling sites. Significant correlation coefficients ( $P < .05$ ) are underlined. Sample sizes of means analyzed are 112 for each summer period.

EARLY SUMMER PERIOD I

<u>BUFO</u>	<u>HYLA</u>	<u>RANA</u>	<u>SHD</u>	<u>TEMP</u>	<u>O<sub>2</sub></u>	<u>DEPTH</u>	<u>MUD</u>	<u>SAND</u>	<u>GRAV</u>	<u>ROCK</u>	<u>OPEN</u>	<u>ISOETES</u>	<u>CAREX</u>	<u>LOG</u>	
1.000	<u>.799</u>	<u>.281</u>	<u>-.243</u>	<u>.718</u>	<u>.157</u>	<u>-.377</u>	<u>.128</u>	<u>.010</u>	<u>-.082</u>	<u>-.214</u>	<u>-.119</u>	<u>.282</u>	<u>.503</u>	<u>.348</u>	<u>BUFO</u>
	1.000	<u>.421</u>	<u>-.465</u>	<u>.589</u>	<u>-.026</u>	<u>-.528</u>	<u>.028</u>	<u>.117</u>	<u>-.035</u>	<u>-.121</u>	<u>.058</u>	<u>.129</u>	<u>.833</u>	<u>.494</u>	<u>HYLA</u>
		1.000	<u>.234</u>	<u>.256</u>	<u>.010</u>	<u>-.261</u>	<u>-.008</u>	<u>.097</u>	<u>.003</u>	<u>-.067</u>	<u>.108</u>	<u>-.081</u>	<u>.488</u>	<u>.610</u>	<u>RANA</u>
			1.000	<u>-.289</u>	<u>.170</u>	<u>.639</u>	<u>.554</u>	<u>-.564</u>	<u>-.436</u>	<u>-.312</u>	<u>-.738</u>	<u>.535</u>	<u>-.638</u>	<u>-.508</u>	<u>SHD</u>
				1.000	<u>.352</u>	<u>-.394</u>	<u>-.102</u>	<u>.138</u>	<u>.073</u>	<u>.037</u>	<u>.129</u>	<u>.007</u>	<u>.389</u>	<u>.266</u>	<u>TEMP</u>
					1.000	<u>.176</u>	<u>.097</u>	<u>-.104</u>	<u>-.084</u>	<u>-.042</u>	<u>-.116</u>	<u>.063</u>	<u>-.185</u>	<u>-.145</u>	<u>OXYGEN</u>
						1.000	<u>.508</u>	<u>-.545</u>	<u>-.397</u>	<u>-.267</u>	<u>-.474</u>	<u>.150</u>	<u>-.726</u>	<u>-.551</u>	<u>DEPTH</u>
							1.000	<u>-.831</u>	<u>-.894</u>	<u>-.594</u>	<u>-.822</u>	<u>.790</u>	<u>-.120</u>	<u>-.264</u>	<u>MUD</u>
								1.000	<u>.816</u>	<u>.147</u>	<u>.715</u>	<u>-.627</u>	<u>.290</u>	<u>.400</u>	<u>SAND</u>
									1.000	<u>.230</u>	<u>.632</u>	<u>-.646</u>	<u>.048</u>	<u>-.314</u>	<u>GRAVEL</u>
										1.000	<u>.583</u>	<u>-.565</u>	<u>-.018</u>	<u>-.087</u>	<u>ROCK</u>
											1.000	<u>-.824</u>	<u>.269</u>	<u>.321</u>	<u>OPEN</u>
												1.000	<u>.004</u>	<u>-.208</u>	<u>ISOETES</u>
													1.000	<u>.574</u>	<u>CAREX</u>
														1.000	<u>LOG</u>

LATE SUMMER PERIOD II

<u>BUFO</u>	<u>HYLA</u>	<u>RANA</u>	<u>SHD</u>	<u>TEMP</u>	<u>O<sub>2</sub></u>	<u>DEPTH</u>	<u>MUD</u>	<u>SAND</u>	<u>GRAV</u>	<u>ROCK</u>	<u>OPEN</u>	<u>ISOETES</u>	<u>CAREX</u>	<u>LOG</u>	
1.000	<u>.588</u>	<u>.122</u>	<u>-.149</u>	<u>.687</u>	<u>.243</u>	<u>-.309</u>	<u>.239</u>	<u>-.087</u>	<u>-.192</u>	<u>-.221</u>	<u>-.244</u>	<u>.341</u>	<u>.339</u>	<u>.161</u>	<u>BUFO</u>
	1.000	<u>.338</u>	<u>-.507</u>	<u>.482</u>	<u>-.130</u>	<u>-.584</u>	<u>-.051</u>	<u>.195</u>	<u>.058</u>	<u>-.116</u>	<u>-.008</u>	<u>.112</u>	<u>.716</u>	<u>.310</u>	<u>HYLA</u>
		1.000	<u>-.253</u>	<u>.170</u>	<u>-.105</u>	<u>-.278</u>	<u>-.085</u>	<u>.247</u>	<u>-.009</u>	<u>-.023</u>	<u>.065</u>	<u>.068</u>	<u>.362</u>	<u>.360</u>	<u>RANA</u>
			1.000	<u>-.289</u>	<u>.226</u>	<u>.651</u>	<u>.507</u>	<u>-.531</u>	<u>-.359</u>	<u>-.230</u>	<u>-.626</u>	<u>.478</u>	<u>-.591</u>	<u>-.324</u>	<u>SHD</u>
				1.000	<u>.361</u>	<u>-.345</u>	<u>-.170</u>	<u>.214</u>	<u>.125</u>	<u>.043</u>	<u>.139</u>	<u>-.054</u>	<u>.362</u>	<u>.183</u>	<u>TEMP</u>
					1.000	<u>.254</u>	<u>.198</u>	<u>-.208</u>	<u>-.138</u>	<u>-.093</u>	<u>-.182</u>	<u>.108</u>	<u>-.236</u>	<u>-.134</u>	<u>OXYGEN</u>
						1.000	<u>.480</u>	<u>-.530</u>	<u>-.339</u>	<u>-.198</u>	<u>-.364</u>	<u>.107</u>	<u>-.620</u>	<u>-.470</u>	<u>DEPTH</u>
							1.000	<u>-.752</u>	<u>-.866</u>	<u>-.541</u>	<u>-.800</u>	<u>.697</u>	<u>-.149</u>	<u>-.297</u>	<u>MUD</u>
								1.000	<u>.750</u>	<u>-.050</u>	<u>.639</u>	<u>-.554</u>	<u>.456</u>	<u>.553</u>	<u>SAND</u>
									1.000	<u>.137</u>	<u>.559</u>	<u>-.577</u>	<u>.022</u>	<u>.323</u>	<u>GRAVEL</u>
										1.000	<u>.534</u>	<u>-.379</u>	<u>-.095</u>	<u>-.177</u>	<u>ROCK</u>
											1.000	<u>-.838</u>	<u>.225</u>	<u>.239</u>	<u>OPEN</u>
												1.000	<u>-.108</u>	<u>-.316</u>	<u>ISOETES</u>
													1.000	<u>.397</u>	<u>CAREX</u>
														1.000	<u>LOG</u>



## APPENDIX III

The selection of a "best" model to describe a data set entails a search of the relative contributions of variables and sets of variables. When variables are intercorrelated, there is no unique, best set of variables, as many different models may provide the same good fit. When intercorrelated, the regression coefficients of any independent variable are imprecise and depend upon which other variables are currently included in the model. Thus, the regression coefficient will not reflect any inherent effect of the particular independent variable on the dependent variable but only a partial effect given which other correlated variables are in the model. In turn, the magnitudes of regression coefficients of variables in the model are affected by the correlated independent variables not included. Also, there is no unique sum of squares that can be assigned to an independent variable as indicating its effect in reducing the total variation in the dependent variable. Any reduction in total variation must be considered in the context of the other independent variables in the model. Stepwise regression techniques are thus inappropriate in analyzing multicollinear data sets and subjective judgements must enter into the search procedure or carefully designed experiments must be utilized to distinguish relevant and nonrelevant variable associations.

## APPENDIX IV

The following table summarizes all substrate preference tests run with Rana cascadae tadpoles. Series I tests were completed in 1977, Series II in 1979. Choice distributions of initial choices and pre-release orientations were analyzed with a binomial test. Substrates and rearing groups tested were bare tank (N), sand (S), gravel (G), and rock (R).

Series	Test	Group	Dev. Stage	Age (Days)	Initial	Choices	P	Pre-release Orientations		P
	<u>N:S</u>				<u>N</u>	<u>S</u>		<u>N</u>	<u>S</u>	
I		N	27	21	15	10	0.424	14	8	0.286
		N	30	47	8	12	0.503	12	7	0.359
		N	31	55	19	6	0.015*	13	8	0.383
II		N	26	8	19	11	0.200	12	13	1.000
		N	33	75	12	8	0.503	6	11	0.332
		N	38	111	18	2	<.001*	12	4	0.077
I		S	27	20	11	14	0.690	12	6	0.238
		S	31	56	21	4	0.001*	15	6	0.078
		S	36	84	16	4	0.012*	9	8	1.000
II		S	26	10	15	15	1.000	8	18	0.076
		S	30	44	17	8	0.108	13	7	0.263
		S	34	74	17	3	0.003*	7	11	0.481
II		G	26	12	13	17	0.585	15	11	0.557
		G	30	44	19	6	0.015*	13	5	0.096
		G	35	76	11	9	0.824	10	9	1.000
II		R	28	28	15	11	0.557	14	10	0.541
		R	29	45	16	9	0.230	10	9	1.000
		R	35	88	17	3	0.003*	12	6	0.238
	<u>N:G</u>				<u>N</u>	<u>G</u>		<u>N</u>	<u>G</u>	
I		N	27	21	20	5	0.004*	15	5	0.041*
		N	31	51	22	3	<.001*	15	7	0.134
		N	36	97	17	3	0.003*	12	6	0.238
II		N	26	11	24	6	0.001*	14	11	0.690
		N	30	46	21	4	0.001*	15	6	0.078
		N	37	103	18	2	<.001*	14	4	0.031*
II		S	26	13	17	8	0.108	10	12	0.832
		S	30	46	19	6	0.015*	16	5	0.027*
		S	36	104	16	4	0.012*	10	7	0.629
I		G	27	22	19	6	0.015*	13	7	0.263
		G	30	51	19	6	0.015*	9	9	1.000
		G	35	97	18	2	<.001*	11	3	0.057
II		G	26	12	19	11	0.200	9	17	0.169
		G	31	48	20	5	0.004*	15	6	0.078
		G	35	106	16	4	0.012*	9	7	0.804
II		R	26	12	15	10	0.424	10	10	1.000
		R	29	45	22	3	<.001*	14	7	0.189
		R	36	104	18	2	<.001*	9	7	0.804

Series	Test	Group	Dev. Stage	Age (Days)	Initial	Choices	P	Pre-release Orientations		P
	<u>N:R</u>				<u>N</u>	<u>R</u>		<u>N</u>	<u>R</u>	
I		N	27	22	19	6	0.015*	14	5	0.064
		N	32	54	20	0	<.001*	13	3	0.021*
II		N	26	16	18	2	<.001*	13	4	0.049*
		N	31	54	18	2	<.001*	11	6	0.332
		N	37	106	17	3	0.003*	12	4	0.077
II		S	26	18	16	4	0.012*	12	7	0.359
		S	30	52	19	1	<.001*	15	3	0.008*
		S	35	101	18	2	<.001*	11	7	0.481
II		G	27	18	18	2	<.001*	8	8	1.000
		G	30	52	17	3	0.003*	12	6	0.238
		G	35	95	18	2	<.001*	11	6	0.332
I		R	27	22	18	7	0.043*	12	9	0.664
		R	31	54	20	5	0.004*	14	5	0.064
		R	36	92	19	1	<.001*	12	1	0.003*
II		R	26	14	22	3	<.001*	8	10	0.815
		R	33	68	18	2	<.001*	9	7	0.804
		R	37	103	18	2	<.001*	13	6	0.167
	<u>S:G</u>				<u>S</u>	<u>G</u>		<u>S</u>	<u>G</u>	
II		N	26	17	21	4	0.001*	19	3	0.001*
		N	32	55	16	4	0.012*	13	6	0.167
		N	36	98	17	3	0.003*	10	4	0.180
I		S	27	23	17	8	0.108	11	9	0.824
		S	30	48	17	8	0.108	15	8	0.210
		S	36	90	12	8	0.503	11	7	0.481
II		S	26	14	11	9	0.824	5	13	0.096
		S	31	55	15	5	0.041*	10	6	0.454
		S	36	96	17	3	0.003*	11	6	0.332
I		G	27	23	15	10	0.424	11	11	1.000
		G	31	48	17	8	0.108	5	13	0.096
		G	35	87	18	2	<.001*	11	7	0.481
II		G	26	15	11	9	0.824	4	11	0.118
		G	31	57	15	5	0.041*	13	5	0.096
		G	36	101	18	7	0.043*	13	9	0.523
II		R	26	18	10	10	1.000	8	9	1.000
		R	32	58	14	7	0.189	10	8	0.815
		R	37	95	16	4	0.012*	13	4	0.049*

Series	Test	Group	Dev. Stage	Age (Days)	Initial	Choices	P	Pre-release Orientations		P
	<u>S:R</u>				<u>S</u>	<u>R</u>		<u>S</u>	<u>R</u>	
II		N	27	19	19	2	<.001*	17	1	<.001*
		N	29	40	20	3	<.001*	11	6	0.332
		N	37	92	17	3	0.003*	10	5	0.302
I		S	27	24	20	5	0.004*	13	4	0.049*
		S	30	57	20	5	0.004*	12	6	0.238
		S	36	82	19	1	<.001*	16	3	0.004*
II		S	27	20	18	2	<.001*	11	4	0.118
		S	30	41	17	3	0.003*	13	5	0.096
		S	36	91	19	1	<.001*	6	10	0.454
II		G	27	23	17	3	0.003*	11	6	0.332
		G	33	68	20	0	<.001*	15	5	0.041*
		G	36	92	18	2	<.001*	11	8	0.648
I		R	27	25	19	6	0.015*	15	6	0.078
		R	31	56	20	5	0.004*	14	7	0.189
		R	35	83	10	10	1.000	8	11	0.648
II		R	27	21	23	7	0.005*	17	11	0.345
		R	30	41	18	2	<.001*	14	3	0.013*
		R	35	89	18	2	<.001*	13	5	0.096
	<u>G:R</u>				<u>G</u>	<u>R</u>		<u>G</u>	<u>R</u>	
II		N	27	23	18	2	<.001*	15	4	0.019*
		N	33	76	20	5	0.004*	13	10	0.678
		N	37	109	17	3	0.003*	11	5	0.210
II		S	27	23	18	2	<.001*	14	3	0.013*
		S	32	70	15	5	0.041*	13	5	0.096
		S	36	89	20	5	0.004*	11	9	0.824
I		G	27	24	16	9	0.230	12	7	0.359
		G	30	49	11	14	0.690	3	14	0.013*
		G	35	91	10	10	1.000	7	8	1.000
II		G	27	19	17	3	0.003*	10	8	0.815
		G	32	75	22	8	0.016*	15	12	0.701
		G	36	111	15	5	0.041*	11	7	0.481
I		R	27	24	15	10	0.424	13	7	0.263
		R	31	48	8	17	0.108	5	17	0.017*
		R	36	90	5	15	0.041*	11	5	0.210
II		R	27	21	22	8	0.016*	15	12	0.701
		R	33	74	13	7	0.263	8	7	1.000
		R	37	110	10	10	1.000	12	5	0.143