ECOLOGY OF ANURAN POPULATIONS INHABITING THERMALLY STRESSED AQUATIC ECOSYSTEMS, WITH EMPHASIS ON LARVAL RANA PIPIENS AND BUFO TERRESTRIS

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ECOLOGY OF ANURAN POPULATIONS INHABITING THERMALLY STRESSED AQUATIC ECOSYSTEMS, WITH EMPHASIS ON RANA PIPIENS AND BUFO TERRESTRIS

presented by

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ABSTRACT

ECOLOGY OF ANURAN POPULATIONS INHABITING THERMALLY STRESSED AQUATIC ECOSYSTEMS, WITH EMPHASIS ON LARVAL RANA PIPIENS AND BUFO TERRESTRIS

By

David H. Nelson

Field and laboratory studies were conducted to determine the responses of anuran populations to thermally stressed aquatic ecosystems. Adult and larval amphibians were sampled in and around a cool arm of a 67 ha reservoir that receives high temperature effluent from a nuclear production reactor on the Savannah River Plant (SRP) in South Carolina.

Adult amphibians were sampled in a mark-release program over a 13-month period by pitfall traps placed peripheral to the reservoir. Species diversity, relative abundance, seasonal occurrence and activity patterns were compared among dominant anurans inhabiting the area. These patterns for some species were compared with data from nearby unheated areas and analyzed in terms of the thermal gradient (16-45 C) extending the length of the reservoir's cool arm.

Larval amphibians were routinely sampled for 15 successive months by dipnets and minnow traps from two stations in the heated reservoir and from five cool seepage ponds flanking the reservoir. All specimens were identified, and larval <u>Rana pipiens</u> and <u>Bufo</u> <u>terrestris</u> were measured for length and staged according to the level of development. In the laboratory, embryonic <u>R</u>. <u>pipiens</u> were reared through metamorphosis to compare survivorship, growth and developmental rates at four constant temperature regimes: 20 C, 25 C, 30 C, and 35 C.

Pitfall trapping studies revealed that three anuran populations dominated (86% of 5,583 captures) the 13 species encountered. Adults and emergent young of the Southern toad (<u>B</u>. <u>terrestris</u>) and the narrowmouthed toad (<u>Gastrophryne carolinensis</u>) were trapped during the same months at the heated reservoir as they were elsewhere on the SRP. Breeding patterns and activity of the leopard frog (<u>R</u>. <u>pipiens</u>), however, were more extensive at the reservoir than at other areas. Adult <u>R</u>. <u>pipiens</u> at the reservoir were active all year, and recently transformed specimens were trapped during 10 months of the year. Captures of migrating adults and juveniles confirm that both <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> bred and developed within protected areas of the heated reservoir. Recapture frequencies ranged from 2% (<u>G</u>. <u>carolinensis</u>) to 16% (<u>B</u>. <u>terrestris</u>).

Although larvae of five anuran species were removed from heated reservoir waters, abundance, species diversity and population density were inversely related to the degree of thermal loading sustained. Having the shortest developmental period, <u>B</u>. <u>terrestris</u> was most successful, although restricted to a single breeding migration yearly. Embryonic and larval mortality in some hot areas exceeded 90%. Within the limits of thermal tolerance, however, growth of larval <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> were both inversely related to temperature and directly related to periods of development. Although all embryonic <u>R</u>. <u>pipiens</u> maintained in the laboratory at 35 C died within nine days, most specimens at 30 C, 25 C and 20 C completed development through transformation. Values for mortality (30-37%) and transformation (63-70%) were similar among the three groups. Corroborating field data, specimens reared at higher temperatures (30 C, 25 C) demonstrated reduced growth rates but increased developmental rates when compared to larvae reared at 20 C. Developmental periods required 6 months (30 C), 8 months (25 C), and >17 months (20 C). Survivorship curves were similar among larvae reared at 20 C, 25 C and 30 C. Specimens demonstrating abnormal development (crooked spine and paralyzed hindlimbs) were significantly greater at 30 C than at 25 C or 20 C. Anomalous development is an apparent manifestation of thermal stress observed only in the laboratory.

Localized cool stream seepage creates isolated microenvironments that allow for marginal development of the most common anurans (\underline{R} . <u>pipiens</u> and \underline{B} . <u>terrestris</u>) in a reservoir receiving supralethal levels of thermal loading (>50 C). The adaptation to breeding during nocturnal rainfall fortuitously confers a double advantage especially to anurans breeding in thermally stressed waters. Both field and laboratory studies confirm that temperatures maintained in excess of 34 C are lethal to eggs, embryos and larvae of most anurans. Within tolerance limits, increased temperatures reduce growth rates but increase development rates. Field data presented here are usually not available for comparison with laboratory populations.

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Although temperature responses must be interpreted at sensitive developmental stages for each species, certain generalities of growth and development apparently hold true within the range of thermal tolerance. It is clear that local physical microhabitats can be altered or effluent temperatures regulated in order to guarantee marginal survival of dominant aquatic populations, and thereby safeguard the stability of the stressed community.

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Вy

David H. Nelson

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INTRODUCTION

National power demands have resulted in increased amounts of heated effluents discharged into natural habitats. The numbers of power plants constructed in this country to meet anticipated electrical demands increases each year (Mihursky and Kennedy, 1967; Summers, 1971). As supplies of fossil fuel decline, nuclear reactors assume more prominent roles of satisfying these demands (Clark, 1969; Levin et al., 1972). The dearth of knowledge on the impact of thermal loading (pollution) as a stress imposed upon the natural flora and fauna presents an immediate practical problem in sound resource and waste management (Cairns, 1972; Mihursky, 1969; Nelkin, 1971). Literature dealing with the effects of thermal pollution has been increasing in recent years (Coutant, 1968, 1969, 1970, 1971; Kennedy and Mihursky, 1967; Raney and Menzel, 1969). Symposia conducted to elucidate some of the major issues (Gibbons and Sharitz, 1974; Krenkel and Parker, 1969), have revealed that little is known about interpreting and predicting responses of stressed populations, communities and ecosystems to thermal loading (Levin et al., 1970). To understand the impact of thermal loading, specific studies are needed to delineate responses of component populations in each affected ecosystem. General concepts based on field and laboratory studies are needed to provide a foundation for maintaining stable community dynamics in such environments. The

aquatic breeding habits of most amphibians subject them to thermal changes in both aquatic and terrestrial ecosystems. Their widespread occurrence in both environments makes amphibians useful indicators of thermal stress.

Studies of temperature adaptation have been conducted primarily at the subcellular, tissue, and organismal levels (Das and Prosser, 1967; Packard, 1972; Prosser, 1958, 1967; Rose, 1967; Troshin, 1967; Ushakov, 1972; Whittow, 1970). Many workers have described differential sensitivities among tissues, organs and systems, as they are affected by temperature. Thus, studies have been oriented mainly towards biochemical, physiological interpretations. Modern terminology and concepts concerning temperature adaptation were presented by Precht (1958) and Prosser (1958), and will not be discussed here. Ecological and physiological considerations are reviewed by Prosser, 1973. Other workers (e g. Brett, 1956; Davenport and Castle, 1895) have reviewed the literature describing rate responses of aquatic forms to temperature. More recent studies delineated maximum thermal tolerances of certain aquatic forms (Bachmann, 1969; Brock, 1970; Moore, 1939; Muto, 1972; Tarzwell, 1970; and Volpe, 1953, 1957a). Some studies involve the determinations of critical thermal maxima (CTM), interpretations of these values, and problems inherent in synergistic agents (Dunlap, 1968; Hutchison, 1961; Seibel, 1970).

Reviewing the literature on thermal acclimation of organisms to high temperature, Davenport and Castle (1895) concluded that acclimatization to higher temperatures occurred across the plant and animal

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kingdoms (in organisms subjected to increased temperature) by loss of water from the protoplasm. They recorded heat rigor in the toad Bufo lenginosus at 40 C to 44 C. Theories about the physiological mechanisms responsible for heat death (Fry, 1967; Heilbrunn, 1956; Hoar, 1966; and Vernberg and Vernberg, 1970) are not discussed in this study. Lillie and Knowlton (1897) presented data of others and their own to demonstrate that growth responses of organisms have optimal levels, and that subminimal and supramaximal temperatures may severely inhibit growth from this optimum. Arrhenius and Van't Hoff devised mathematical formulae to describe the Q_{10} relationship between temperature and chemical reactions (Belehradek, 1935). Krogh (1914) showed that temperature influenced embryonic development of a fish, frog, water beetle, and sea urchin in proportion to the temperature increment. Crozier (1926) using a variety of plants and animals, Atlas (1935), Bachmann (1969), Grainger (1959), Hoadley (1938), and Ryan (1941) described growth or developmental phenomena in relation to temperature.

Water temperature is generally regarded as the most critical environmental factor to which anurans are exposed during breeding and embryonic development. Development of anuran eggs and larvae have been shown to be directly related to water temperatures (Herreid and Kinney, 1967; Moore, 1939, 1942a; Zweifel, 1968). Thermal tolerances and developmental rates of anuran eggs, embryos and larvae have been correlated with breeding habitat, season, and geographical distribution (Ballinger and McKinney, 1966; Licht, 1971; Moore, 1939, 1949; Ruibal, 1957; Volpe, 1953, 1957a).

The effects of thermally stressed aquatic environments on normal growth and developmental processes are difficult to evaluate. Laboratory studies demonstrating thermoregulation in larval amphibians (reviewed by deVlaming and Bury, 1970) are often difficult to relate to the more complex natural conditions. Moreover, field data on thermoregulatory behavior of larval amphibians are scanty (Brattstrom, 1962, 1963).

Common problems arise from attempts to formulate generalities which allow comparisons of organisms, populations and ecosystems. Because of its complexity, workers have approached the problem of thermal stress from various perspectives. The conclusions are often confounded by factors such as synergism, acclimation, and variable thermal responses among different age groups. Although some field studies conducted revealed negligible effects of heated effluent (Alabaster, 1964; Merriman, 1970), others (Jones, 1964; Trembley, 1960) substantiate serious damage. Additional studies are needed (Levin <u>et</u> <u>al.</u>, 1970) in different habitats in different regions of the country.

In the present study, laboratory and field studies were designed to characterize population responses of larval anurans (frogs and toads). To determine how thermally stressed aquatic environments evoke population responses in field situations, adult and larval amphibians were sampled along the periphery of a reservoir receiving lethal levels of heated effluent from a nuclear production reactor. Cool shoreline seepage in some parts of the reservoir created isolated microenvironments marginally habitable to amphibian larvae. Responses to stress

were characterized in terms of larval survivorship, growth and development. To compare the success of amphibian populations occurring in the vicinity of the heated reservoir and determine the range of thermal conditions that they can tolerate, the level of thermal loading was related to several variables. The numbers of species occurring around the reservoir, and those breeding, developing, and emerging were related to thermal levels in the different areas.

Another goal was to determine the extent to which an aquatic ecosystem can be thermally stressed before it is rendered uninhabitable for naturally occurring amphibians. A basis was sought for predicting the maximum thermal limits that can be safely imposed on the Southeastern reservoir studied, yet allow amphibian development. Hazards (\underline{e} \underline{g} . thermal stress, and modifications in food availability, cover and predation) related to the direct thermal influence were also of interest.

Prominent population responses should be demonstrable both in the field and laboratory. Consequently, in addition to field studies conducted in the thermally stressed reservoir habitats, larval <u>Rana</u> <u>pipiens</u> Schreber were reared in the laboratory at four constant temperature regimes: 20 C, 25 C, 30 C and 35 C. Survivorship, growth, and developmental rates of laboratory reared larvae were compared among the thermal regimes and the laboratory findings compared to those observed in the field.

To achieve these goals, four distinct, yet related, programs were pursued (three in the field and one in the laboratory):

- 1. Post-larval anurans were captured using a drift fence-pitfall trap method throughout the year to monitor seasonal occurrence and movements (localized activity and migrations). The pitfall trapping study was designed to indicate which anurans occur in the vicinity, and thus provide a listing of potential reservoir breeders. The occurrence of recently metamorphosed emergent transformees would also provide evidence of which species successfully completed larval development in the reservoir. Due to placement of pitfalls peripheral to the thermal gradient of the reservoir, both adult breeding migration to and emergence of transformed young from the reservoir would become evident. Seasonal activity and movement patterns were to be related to the thermal gradient and to the location of associated cool seepage ponds flanking the reservoir.
- 2. After establishing which amphibians were active around the reservoir, a sampling program was undertaken to document which (if any) anurans deposited eggs, and survived through embryonic and larval development there. Samples were systematically removed monthly from both heated and cool areas in the vicinity of the reservoir by dipnet. Minnow traps were also maintained in cool and heated aquatic environments for 11 months. Data obtained from these studies were to provide evidence for the presence or absence of larval anurans in different thermal regimes along the thermal gradient. Abundance and species diversity of larval amphibian populations were to be compared

among cooled shallows of the heated reservoir and peripheral stream seepage ponds.

- 3. In the third field study, larvae of the Southern toad (<u>Bufo</u> <u>terrestris</u> Bonnaterre) developing from eggs naturally deposited in the reservoir were sampled from cool and heated areas. Growth and developmental responses of a single breeding migration of <u>B</u>. <u>terrestris</u> in March 1972, were then compared among several subpopulations in aquatic habitats receiving variable levels of thermal loading. Breeding activity in the thermal waters provided an excellent opportunity to compare growth and developmental rates among larvae at different temperature regimes in a non-laboratory situation.
- 4. An experimental rearing of <u>Rana pipiens</u> was conducted in the laboratory. Embryos from the field were maintained at four constant temperatures to allow for a comparison of growth and development at different thermal regimes. Manifestations of thermal stress evident among larvae sampled in the reservoir were compared with those observed under laboratory conditions.

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MATERIALS AND METHODS

Description of the Study Area

All field studies were conducted along the periphery of a 67 ha reservoir ("Pond C") formed in 1958 to receive heated effluent from a nuclear production reactor on the Savannah River Plant near Aiken, South Carolina. Temperatures of the reservoir are classified as "Secret" information and only those relevant to the ecological studies are reported. Heated effluent is carried to the reservoir through a thermal canal. After entering the reservoir, thermal effluent passes down the main channel (Fig. 1) and thereafter into another reservoir: Par Pond. Since the current does not flow directly into the cool arm of Pond C Reservoir, temperatures there consist of a thermal gradient which was divided into six thermal zones for the present study (Fig. 2). At the mouth of the cool arm (zone VI), water temperatures may approach 50 C and exceed the thermal tolerance of most organisms with the exception of thermophilic bacteria and bluegreen algae. Temperatures are considerably reduced at the opposite extreme of the thermal gradient (zone I, Fig. 2). Incurrent stream flow there allows water temperatures at times to ^aPProach levels typical for this region of the country. Most common anurans can be collected or heard calling there in season.

Fig. 1. Pond C Reservoir on the Savannah River Plant, South Carolina. Letters represent locations of drift fence-pitfall trapping areas along the periphery of the reservoir's cool arm. Arrows indicate the direction that heated effluents flow from the thermal canal through the reservoir.



Fig. 2. The cool arm of Pond C Reservoir. Pitfall trapping areas are grouped into thermal zones along the thermal gradient. Temperatures represent the mean minimum and mean maximum (N = 15) for March 1972. Seasonal temperature data for the six zones are given in Table 1. Seepage ponds are apparent near sites A, B, I, J, and Z.



The reservoir was constructed over a natural creek bed. The water table along most of the reservoir, therefore, is very close to the surface of the ground. Stream seepage is evident in low-lying areas throughout the length of the cool arm. Several cool seepage ponds occur contiguous to the reservoir (Fig. 2). All five ponds (except the one near site Z) maintain readily apparent and steady flow of the underground water seepage into the reservoir, and may on occasion contact the reservoir proper. The terrestrial habitats surrounding the reservoir are characterized by mixed hardwood and pine of the "Oak-Pine Forest Region" (Braun, 1964); the soil type is predominately sand.

Field Studies

Pitfall Trapping Survey

Beginning at the terminal pool (upstream end) of the cool arm of the reservoir, pitfall-drift fence sections were laid at regular intervals on both sides (Fig. 1). Thirteen sections were placed along the eastern periphery of the reservoir at intervals of 100 m. On the west side, six additional pitfall-drift fence sections were spaced at intervals of 200 m. Thus, traps were distributed throughout the length of the thermal gradient. Habitats ranged from low, marshy areas to high, xeric ones and from open grasslands to dense woods.

Traps were situated as close to the water as practical (2-5 m), although a high water table or elevated terrain in some areas necessitated placement of cans farther away from shore. The traps sampled more than 2.2 km of shoreline along the thermal gradient. Each trap consisted

of four pitfalls (cans 42 cm deep and 35 cm in diameter), and 15.2 m of aluminum flashing (51 cm high). Flashing was buried 4-5 cm below the soil level and fastened with wire to 1.3 m metal stakes driven into the ground (Fig. 3). A 10 m section of drift fencing was laid in a straight line with a bucket (pitfall) buried at each end of both sides, flush with the soil surface. Holes were punched in the bottom of pitfall cans to allow for drainage of rain. A 1.3 m section of flashing extended beyond each bucket on both sides, at each end of the drift fence at 60° angles (Fig. 4). Amphibians, reptiles and other small animals encountered the drift fence and followed it to the pitfalls at either end.

Frequency of field visits during the year varied with the catch. In the spring, traps were usually checked daily; in the summer, every 2-3 days; and in the winter, every 3-4 days. Travel between traps was by boat, as most drift fences were not readily accessible by road. Amphibians were removed from cans and the following data recorded: Species, snout-vent length, sex, and whether the individual was new or a recapture. Each specimen was toe clipped and released outside the drift fence lateral to the pitfall from which it was removed. The outside toe on the right forelimb was removed to allow discrimination of initial captures and subsequent recaptures. Throughout the duration of the study, maximum-minimum thermomenters were maintained along the shoreline of the thermal gradient. Data were recorded every 2-3 days.

Fig. 3. End view of pitfalls. Two cans were buried at each end of the drift fence.



Figure 3
Fig. 4. Drift fence. Organisms encountering the fence follow it and move towards the terminally placed pitfalls.

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<u>Reservoir Sampling with Dipnets and</u> <u>Minnow Traps</u>

Since the presence of larvae would confirm successful breeding and development, several areas were selected for a monthly sampling program: cool seepage ponds A, B, I, Z and heated reservoir shorelines V and K (Figs. 2, 5, 6, 7, and 8). During the latter part (25-30 days) in each month (June 1971 through July 1972), the sites selected were routinely sampled using two dipnets: a small mesh Turtox Bueno model and a large 6 mm mesh nylon net. Sampling periods at each site lasted for a minimum of 20 minutes and continued thereafter until no new species were encountered. Specimens were immediately placed in 150 ml of 10% formalin. To insure proper preservation of specimens, these solutions were changed later the same day and one week thereafter. Larvae were brought into the laboratory for identification, using Altig's (1970) key, measurement (total length to nearest 0.2 mm), and staging according to the level of development (Gosner, 1960).

Two locations in the reservoir were regularly sampled: near pitfall sites V and K (Figs. 7 and 8). Both received cool stream seepage that usually reduced the water temperature in the shallows 10-20 C below those of nearby shores. The gradually sloping shoreline at site V contained shallows ranging from a few millimeters to 0.3 m. It contained emergent vegetation (mostly <u>Scirpus americanus</u>) for a distance of approximately 40 m. Site K, approximately 600 m closer to the hotter end of the thermal gradient, was the warmest area sampled routinely. Emergent vegetation there was restricted to a small stand of cattail (<u>Typha latifolia</u>). Other reservoir areas receiving seepage were sampled periodically.

Fig. 5. Cool seepage pond A. This cool pond is located near pitfall trapping area A (Fig. 2). The cool arm of the heated reservoir is shown in the background.

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Fig. 6. Cool seepage pond I. This cool pond is located near pitfall trapping area I (Fig. 2). Shoreline seepage is evident in the foreground and drainage into the reservoir can be seen in the background.



Fig. 7. Reservoir shoreline V. The shallow shoreline of Pond C Reservoir is near pitfall trapping area V (Fig. 2). Cool seepage here reduces shoreline temperatures to levels habitable to larval amphibians.

n 2



Fig. 8. Reservoir shore K. This reservoir site (near pitfall trapping area K) was the hottest area sampled routinely for larval amphibians. Shoreline seepage, evident in the foreground, reduces water temperatures to allow for marginal survival of anuran larvae there. Both <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> bred there; both incurred high mortality. (A minnow trap is present in the water just behind the log.)



Figure 8

The seepage ponds chosen for routine sampling were located near pitfall sites A, B, I, and Z (Fig. 2). Additional sites were sampled periodically for comparison. Pond designations represent the names of pitfall trap sites to which they are nearest. The association of seepage ponds with the reservoir ranged from a continuously flowing stream connection (B) to total separation (Z). Pond I was connected to the reservoir at high water levels only (mostly spring and winter), and Pond A was rarely ever continuous with the reservoir.

To supplement the monthly sampling program, three to four minnow traps were maintained in each of the six locations mentioned (from 3 September 1971 to 2 August 1972). Other areas were also sampled with minnow traps from time to time. During each field visit to the pitfalls, the minnow traps were checked also. The specimens caught were removed from traps and recorded by species. Anuran larvae were staged and measured in the field, or preserved and taken to the laboratory for staging and measuring.

Reservoir Sampling of Larval Bufo terrestris

Large numbers of larval <u>Bufo terrestris</u> were encountered on 29 March 1972, in cool seepage ponds flanking Pond C Reservoir and along the shallow shoreline in the cooler parts of the reservoir proper. Swarming masses of larvae were numerous in cooler reservoir shallows (3-10 cm deep) within 0.5 m of the shoreline, venturing into deeper, warmer water only if provoked from the shore. Larval densities were noticeably reduced or absent in shoreline areas with little or no seepage, sparse vegetation, and thus, high temperatures.

On 29 March, samples were taken from several locations in the heated reservoir and from cool, peripheral seepage ponds to compare growth rates and development of larvae from different thermal regimes. During the following three weeks, successive samples were removed weekly from sites where population densities were sufficient. Sampled areas were separated by thermal barriers of open shore without seepage or vegetation. Increased temperatures (5-10 C above ambient) probably precluded movement along most of the shoreline, thus maintaining the integrity of sampled "populations."

Each sample of larval <u>B</u>. <u>terrestris</u> (30 specimens, if available) was removed with a fine mesh dip net and preserved in the field. Samples were taken to the laboratory where they were identified (Altig, 1970), measured (total lengths), and staged (Gosner, 1960) according to the level of development. In an initial analysis, ten larvae were examined from each sample. Since these data indicated trends in growth and development, 20 additional specimens were examined from areas where sample sizes permitted. For the supplemental analyses, the staging system illustrated by Limbaugh and Volpe (1957) was adopted. The thermal ranges (given in the text) represent the daily mean minimum and daily mean maximum temperatures recorded at each study site during the four week period of study.

Sufficient numbers of larvae were available for three consecutive samples at sampling areas B, A and V (Fig. 9). Area B (a cool pond, peripheral to the reservoir) receives a constant flow of underground stream seepage and is continuous with the reservoir only at high water

Fig. 9. Sites at the reservoir from which larval populations of <u>Bufo terrestris</u> were sampled. Areas A, D, V, and K were along the shore of the heated reservoir; area B was a nearby cool seepage pond. Temperatures represent mean maximum recordings over periods of two to three days (n = 15) during March 1972.



Figure 9

levels. Temperatures there ranged from 8 to 24 C. The pond (B) was completely separated during the four weeks of the sampling period; thus, it received no thermal effluent. Area A was located at the cooler end of the thermal gradient in the reservoir proper where temperatures ranged from 21 to 33 C. Compared to area V, area A received "moderate" thermal loading.

Area V (Fig. 7) was the warmest area (27-34 C) from which successive samples were available and was 350 m closer to the thermal inflow than thermally intermediate area A. Extensive cool shallows in the reservoir at area V served as thermal refuge areas for certain aquatic forms (invertebrates, fish and amphibians) not found in the reservoir proper. The shore at area V was very gradually sloped, with a pool (2.2 m in diameter and 0.5 to 1.0 m deep) that always contained water. When reactor flow was temporarily interrupted, the reservoir water level fell 0.3 to 1.3 m, completely exposing 1-8 m of the previously submerged shoreline. Then only the pool area remained, surrounded by dry land.

Although successive samples were unavailable, larvae were removed from heated areas D (28-35 C) and K (29-36 C; Fig. 9). Area D received little seepage, thus larvae did not have access to cool shallows as in area V. Area K (Fig. 8) was the hottest site from which larval amphibians were ever collected. It received cool seepage, and was partially protected from lethal reservoir temperatures by a shallow sand bank between the pool and the reservoir.

Larval condition (length and stage of development) can be considered as a function of age and temperature. If larvae were the same age (representing a single breeding migration), any significant difference in growth and development could be attributed to temperature. Two major questions were of concern regarding growth and development. First, do they vary as a function of thermal regime? To test whether developmental stages and lengths were the same at three thermal regimes (areas A, B and V), specimens were compared weekly with the Kruskal-Wallis nonparametric analysis of variance (Sokal and Rohlf, 1969). The null hypothesis was that the three populations were at comparable stages of developmental stage varied among the different thermal regimes. Lengths at comparable stages of development were pooled among sampling areas and tested with the Kruskal-Wallis nonparametric analysis of variance.

Laboratory Rearing of Larval Rana pipiens

Sixteen 20 gallon (84.6 1) aquaria, placed on metal stands, were employed: four replicate aquaria in series at each temperature (Fig. 10). Each tank was originally filled with 84.6 1 of water from Par Pond on the Savannah River Plant. Evaporation was compensated by the addition of (glass) distilled water back to the original level. To reduce temperature fluctuations within the aquaria, the four sides of each were covered with fiberglas insulation. Covers made of 1.2 cm plexiglas rested flush on the top of each tank. The insulation was found to

Fig. 10. Experimental apparatus employed to rear embryonic <u>Rana</u> <u>pipiens</u> through metamorphosis in the laboratory. Four replicate aquaria were maintained at each of the four temperatures: 20 C, 25 C, 30 C, and 35 C.



Figure 10

reduce temperature fluctuations appreciably at the warmer thermal regimes, and the plexiglas covers considerably reduced evaporation and stabilized temperatures in the aquaria. At the beginning of the study, builders sand was soaked and rinsed for 24 hours in running water, then placed to cover the lower 2 cm of each aquarium bottom. On 16 December 1971, the sand in each tank of the remaining thermal regimes was removed and replaced with gravel and undergravel filters weighted down by two large rocks (Fig. 11). Rocks were later replaced with small culture dishes.

Water was continuously circulated among the four replicate aquaria at each temperature. Air lift pumps were employed to move water from the bottom of one tank to the surface of the tank next in the series (Fig. 10). Excelon (polyurethane) tubing (1.9 cm in diameter), 18 ga. syringe needles, and bent sections of 8 mm (0.D.) pyrex glass tubing were used. Air pressure was supplied by a 3/4 H.P., two cycle oilless Bell & Gossett air compressor, set to deliver at a pressure of approximately 5 psi. To maximize efficiency of air lift pumps, the water column was lifted from the floor (95-98 cm). This system was found to circulate water at an average rate of 0.6 l/minute. Interconnecting, inverted U tubes of 13 mm (0.D.) pyrex glass tubing were also placed as siphons among the four aquaria in each series. These prevented water overflow created by differential transfer rates of water by the four different air lift pumps.

Fig. 11. View of larval <u>Rana pipiens</u> kept at 20 C. Rocks weighing down undergravel filters were later replaced with glass culture dishes. Considerable size differences among larvae indicated highly variable growth rates at 20 C.

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To assess effects of continual thermal loading on larval growth and development, embryonic <u>Rana pipiens</u> were reared at different thermal regimes. On 2 September 1971, two egg masses were collected among dense shoreline vegetation of Dick's Pond, a 0.97 ha farm pond on the Savannah River Plant, 6.6 km from Pond C Reservoir. The embryos were carefully separated in the laboratory, staged, divided into groups of 100, and placed in culture dishes half-filled with pond water at 18 C. Each group was counted twice; only normal embryos were included. Only nine dead or distorted embryos were found among the approximately 2,000 eggs examined. A group of 100 embryos was introduced into each of the four series of aquaria at each constant temperature regime (20 C, 25 C, 30 C, 35 C). Groups of the more advanced cohort (stage 18: "muscular response," Gosner, 1960) were placed in the last two tanks (C and D) at each temperature. The less advanced group (stage 11: "mid-gastrula") was placed in the first two tanks (A and B).

To prevent movement of embryos by circulating water currents, the eggs were temporarily placed inside 26 ga., stainless steel screen cylinders (approximately 21 cm in diameter), placed upright in the center of each tank. Embryos remained inside the screen cylinders from five to seven days, until reaching the first larval, feeding stage (26). At that point the screen cylinders were removed and larvae became distributed throughout the aquaria. Barriers of stainless steel screen were placed around the heaters and interconnecting siphons to reduce experimental mortality of larvae. To keep larval amphibians from being transferred from one aquarium to the next by the air lift pumps, or

interconnecting U tube siphons, the entrances to both were surrounded by cylindrical siphon covers. Each of these cylinders was made from 36 ga. stainless steel screen, and measured 4-6 cm in diameter. Fiberglas insect screening was used to bind the bottom of the siphon cover. It was attached to the stainless steel with nichrome wire.

Water was continuously circulated through outside filters, driven by air lift pumps. Originally four liter jars containing charcoal and glass wool were used. As the growing larvae began to eat more, and deposit greater amounts of fecal debris (on 16 December 1971), more efficient metaframe outside filters were adopted. At the same time, undergravel filters were also installed. The sand was removed from each aquarium, and gravel was placed over the undergravel filters. First rocks, then later culture dishes were placed over each side of the undergravel filters to prevent their rising. Since tadpoles demonstrate a preference for "close quarters," they tended to collect in corners along the air stands of undergravel filters, behind siphon covers, at the end of the aquarium, and under leaves of spinach. For this reason, precautions had to be taken to prevent larvae from working their way below the undergravel filters. Mortality of several larvae resulted from such an occurrence. They died below the undergravel filters, apparently unable to get out.

Constant temperatures were maintained by "Jumo" (Preiser) mercury thermoregulators, relay boxes (Precision Temperature Regulator, Eastern Industries), and heaters prepared in the laboratory (Fig. 10). The relay boxes automatically turned on heaters as the temperature fell

below that for which the thermoregulator had been set. They automatically switched the heaters off when the thermoregulator was restored to the temperature for which it was set. Temperature fluctuations rarely exceeded 1 C in 24 hours. Since each temperature series was supplied with a single thermoregulator, any difference in heaters would perpetuate a warmer or cooler temperature in certain tanks. Whereas an identical heater was required for each aquarium to reduce temperature variation, they were constructed from glass tubing, nickel-chromium wire, sand, and insulated wire, and connected in parallel.

An efficient heater, in order to maintain temperature constancy, should at best be working half of the time for maximum efficiency. This heating rate reduces the time lags that result from on-off switched systems and the accompanying thermal fluctuation. In order to increase the heating surface of the heaters, 13 mm (0.D.) pyrex glass tubing was bent into a U-shape, with dimensions (28 cm wide x 33 cm high) appropriate to fit into the end of each aquarium. Twenty feet (6.1 m) of nichrome wire was coiled and placed into the lower 17.8 cm of the U, and strained sand placed around the heating coil as insulation. Water circulated from one aquarium to the next; it was withdrawn from the bottom of the cool end and deposited at the surface of the end in which the heater was positioned. This system of circulation (in addition to the water agitation created by undergravel filters) prevented thermal stratification within tanks.

To insure that the room temperature did not exceed the minimal temperature regime of 20 C, an air conditioner was constantly run and generally maintained a temperature of 17 to 19 C. A cool room temperature obviated the need for other mechanisms to reduce temperatures. Temperatures were checked daily at 0900 and 2100 hours for the first two weeks; observations of embryonic conditions and counts were recorded each time. Thereafter, temperatures and accounts of larvae in each tank were recorded once at each daily check. The upper and lower diel temperature fluctuations were recorded for each temperature treatment by maximum-minimum thermometers. They provide a profile of minor fluctuations. Except for two major power failures, totalling less than 29 hours, daily temperatures did not fluctuate more than ±1 C.

In order to provide a uniform source of lighting, the windows in the laboratory were covered with aluminum foil. All illumination came from the eight fluorescent ceiling lamps. A uniform periodicity, a 12 hour light-12 hour dark diel cycle, was employed throughout the study with the aid of an electric timing switch. The plexiglas covers on aquaria allowed uniform illumination to each.

Frozen spinach was fed ad libitum. For the first 10 months of the experiment, freshly thawed spinach was added daily. Thereafter (when a few larvae remained at 20 C only) spinach was added every other day. Decomposition of spinach at 20 C was very slow. Food was not allowed to remain inside an aquarium for more than two days. At each feeding, old spinach was removed with a hand net. Any spinach or fecal debris was thoroughly removed with fine mesh nets, and the new spinach

placed on top of the undergravel filters, covered with pieces of gravel to keep it weighted down.

Daily procedures involved recording of temperatures, feeding, cleaning, measuring and staging specimens, and recording data on dead or transforming larvae. Charcoal and glass wool in outside filters were changed one to three times a week as necessary. At each checking, temperatures at the bottom, cool end were recorded for each tank. Data provided by a maximum-minimum thermometer in each thermal regime provided a constant record of temperature variation.

Dead specimens were recorded, measured, staged and preserved when discovered. Any abnormalities were noted. All larvae removed were preserved in a 7.6 X 2.5 cm vial in 10% formalin and stored for a double checking of data. The formalin was replaced within two weeks with a fresh solution to assure proper preservation.

Statistical Treatment

Certain statistical conventions are consistently employed in the text, figures and tables. The total sample sizes are represented by "N"; the numbers of observations in subsamples are represented by "n." The standard error of the mean (SE) is the measure of variance most commonly employed although the range is occasionally given. The designation "CV" represents the coefficient of variation.

The original experimental design called for a comparison of larval growth and development as a function of the major covarients temperature (thermal regime) and age. The same analysis was chosen

for larval <u>R</u>. <u>pipiens</u> reared in the laboratory and for larval <u>B</u>. <u>terrestris</u> sampled weekly from the reservoir. In preliminary examination, data sets were analyzed for variance homogeneity and normality (Bartletts' test and Kolmogrof-Smirnoff test: after Sokal and Rohlf (1969). All data sets were heteroscedastic--uncorrected by transformations (log, square root, square, and reciprocal). About half of the data sets were normally distributed. Since the assumptions of parametric statistics could not be satisfied, data were compared with the Kruskal-Wallis nonparametric analysis of variance and chi square tests.

RESULTS

Field Study: Pitfall Trapping Survey

A bimonthly thermal profile of mean minimum and mean maximum temperatures was recorded in arbitrarily assigned zones along the cool arm of the reservoir, and attests to the seasonal perpetuation of a thermal gradient there (Table 1). Nine species of urodeles (salamanders) and 13 species of anurans (frogs and toads) were collected in pitfall traps over the 13 month period (Table 2). The purpose of the study was to focus on the major amphibian populations breeding in the reservoir, hence further consideration will be limited to the five dominant anuran species for which sample sizes were larger. The numbers of salamanders trapped were low in comparison to frogs and toads.

Of 1,784 <u>Rana pipiens</u> trapped, 301 (17%) were recaptured (Table 3). The majority of specimens (63%) were caught from May through July. To compare size classes, the ranges of body lengths are presented for monthly total captures. Adult specimens exceed 49 mm and emergent young range from 20 to 33 mm (Wright and Wright, 1949). Although adults were found to be active throughout the year, emergent transformees were trapped every month but February, 1972.

The low mean body lengths of initially captured specimens from March to July (smaller than those for total captures or recaptures),

Table 1. Mean maximum (upper) and mean minimum (lower) temperatures (n = 14) recorded bimonthly in the six thermal zones of Pond C Reservoir and in cool seepage pond A (Fig. 2). The zones represent the thermal gradient that occurs in the cool arm of Pond C Reservoir on the Savannah River Plant in South Carolina

	Cool			Therma	1 Zone		
	A	I	II	III	IV	۷	VI
February	16.2	27.8	32.3	32.7	33.4	34.1	35.8
	5.8	17.3	25.2	26.1	26.4	27.1	28.5
April	29.0	34.1	38.0	37.9	38.9	39.6	41.1
	12.4	26.8	31.0	32.9	32.2	33.1	35.0
June	33.3	39.7	42.6	43.0	43.3	44.4	45.2
	21.3	33.1	35.8	38.0	37.9	39.1	40.1
August	38.9	37.7	40.6	40.5	40.9	41.5	42.3
	24.3	33.7	34.6	36.3	36.3	37.4	38.1
October	23.5	35.3	39.8	40.4	41.0	42.1	43.2
	17.7	29.4	34.4	36.3	36.3	37.5	38.7
December	18.8	29.4	32.5	33.0	33.7	34.0	36.2
	9.2	20.8	25.0	27.7	28.1	27.5	30.3

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Species ^a	Feb ^b	Mar	Apr	May	ղոր	ามก	Aug	Sep	0ct	Nov	Dec	Jan	Feb	Mar	Total
R.pipiensB.terrestrisG.carolinensisA.gryllusP.triseriataR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaR.catesbeianaB.ornataMonthly totals	10 10 10 10 10 10 10 10 10 10 10 10 10 1	147 259 0 13 7 0 0 427 0 0 0	169 151 151 151 151 150 169 164 164	500 81 81 1 20 20 20 20 20 20 20 20 20 20 20 20 20	400 203 323 323 43 43 43 5 21 23 33 8 0 0 0 0 0 0	241 85 396 15 13 13 13 792 792	55 50 33 33 33 50 0 0 0 0 0 39 1 50 50 50 50 50 50 50 50 50 50 50 50 50	40 75 75 75 75 75 75 75 75 75 75 75 75 75	57 105 105 230 230 230	83 83 83 83 83 83	28 14 58 00 28 28 28 28 28	4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1 34 00000000 34 00000000 34 00000000 34 00000000	19 653 8 19 19 10 706 706	1,798 1,641 1,641 1,369 272 272 173 173 173 173 173 546 546 546 5583 5583 5
P.glutinosusN.viridescensM.quadridigitatP.montanusD.fuscusE.bislineataE.longicaudaP.ruberMonthly totals	200000 N	0,004-0000	26 32 32 00000 13 26	84400000 <u>44</u>	38 55 0 0 0 3 0 3 4 7 5 5	v−000000	w-4000000	260010002440	00 10 10 10 10 10 10 10 10 10 10 10 10 1	∞0400- <u> </u> [15 32 32 0 - 2 - 4 - 0 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	0 10 10 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	∞ <u>0000</u> -0002	4-0-00-00	146 45 45 43 43 19 17 17 291
^a Anuran genera in order: Eurycea, Eurycea	genera: Pletho , and Ps	<u>Rana</u> , don, <u>N</u> eudotr	Bufo lotoph iton.	, <u>Gas</u> thalm	trophry Js, Man	culus	cris, Pse	Pseu	i ton,	Amby	aphio stoma	, Des	<u>Hyla;</u> mogna	Urod thus,	ele

^bData available only for 26-28 February.

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		Total (Captures		Init	ial Capt	tures		Recaptun	es
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February ^a	99	36.2	0.84	29-58	64	34.1	0.54	2	32.0	:
March	147	33.8	0.60	25-67	129	31.8	1.05	18	33.8	1.58
April	169	34.0	0.33	27-60	140	33.4	0.66	29	32.4	0.48
May	496	33.0	0.22	21-66	388	32.5	0.57	108	32.5	0.44
June	393	32.6	0.32	20-68	342	32.0	1.57	51	33.0	0.76
July	241	35.0	0.42	23-68	209	33.6	1.77	32	36.7	0.79
August	55	42.2	1.04	28-68	45	38.2	3.74	10	44.0	0.47
September	38	44.2	1.75	25-61	29	34.0	9.64	6	46.0	1.88
October	57	40.6	1.90	20-74	49	29.5	5.69	8	49.2	4.91
November	24	46.4	1.98	30-60	17	34.3	5.72	7	49.0	3.12
December	28	48.5	2.54	25-75	24	32.0	4.80	4	49.8	3.17
January	40	48.0	1.75	26-63	33	31.5	1.69	7	55.4	4.09
February	11	55.8	1.17	50-62	0	;	ł	11	55.8	1.17
March	19	47.0	2.71	23-72	14	31.6	6.15	2	48.8	1.46
	1,784				1,483			301		

^aData available only for 26-28 February.

indicate that emergent young comprise the majority of specimens trapped then. The percentage of specimens recaptured increased during the first four months to 22% of the total specimens captured in May. Except for February 1972, when all 11 specimens trapped were recaptures, the highest monthly recapture rate was 29% (November). Mean body lengths of immature (< 47 mm) <u>R</u>. <u>pipiens</u> (Table 4), verify that emergent transformees dominate monthly captures for most of the year. A biweekly analysis of trapping data reveals a highly significant correlation between the mean body length and number of <u>R</u>. <u>pipiens</u> trapped biweekly (Spearman r = -0.813, P < 0.0001).

<u>Bufo terrestris</u> showed a distinctive bimodal seasonal activity pattern. The periods of greatest capture were March-April and June (Table 5). Emerging transformees (5-10 mm) were encountered only in May, June and July. No <u>B</u>. <u>terrestris</u> were caught from November to January. A total of 272 (17%) of the individuals were recaptured.

<u>Gastrophryne carolinensis</u> (the narrow-mouthed toad) was trapped from April to December (Table 6) demonstrating a bimodal seasonal activity pattern. Most of the specimens (87%) were trapped from June through September, and only 3% were recaptured. Emergent young (10-13 mm) were trapped from July to October.

The numbers of the southern cricket frog (<u>Acris gryllus</u>) trapped monthly also represent bimodal seasonality (Table 7). Emergent young (10-15 mm) were trapped from July to December. Numbers of specimens trapped were much fewer than those of <u>R</u>. <u>pipiens</u>, <u>B</u>. <u>terrestris</u> or <u>G</u>. <u>carolinensis</u>. Only eight (3% of 271) were recaptured.

Month	N	X Length	SE	Range
February ^a	59	34.02	0.273	29-39
March	132	31.77	0.291	25-46
April	159	33.23	0.216	27-42
May	473	32.38	0.156	21-43
June	383	32.10	0.274	20-44
July	223	33.97	0.331	23-46
August	40	38.82	0.831	28-46
September	18	34.78	1.708	25-44
October	33	30.12	1.157	20-44
November	8	34.12	1.597	30-43
December	10	32.90	1.703	25-41
January	9	31.56	0.973	26-36
February	0			
March	9	33.11	1.540	23-40
	1,556			

Table 4. Numbers and body lengths (mm) of immature <u>Rana pipiens</u> trapped in pitfalls along the periphery of Pond C Reservoir (1971-1972)

^aData available only for 26-28 February

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		Total (Captures		Initi	ial Capt	tures		Recaptur	,es
	z	X	SE	Range	z	Х	SE	z	Х	SE
February ^a	10	52.9	0.87	49-57	10	52.9	0.87	0	1 1	1
March	256	53.7	0.34	45-83	216	53.8	0.38	40	53.2	0.78
April	248	50.3	0.19	27-77	174	50.5	0.58	74	49.7	0.54
May	81	37.1	1.57	6–60	69	35.1	1.71	12	48.2	1.98
June	201	37.8	1.28	5-75	165	35.4	1.46	36	48.7	1.44
յսլչ	85	40.4	1.28	10-61	68	38.5	1.41	17	48.1	2.16
August	50	43.8	0.95	35-63	37	42.0	0.87	13	48.8	2.16
September	24	48.5	1.86	30-62	13	48.8	2.86	11	48.2	2.40
October	17	50.0	2.23	28-61	10	50.1	3.73	7	49.8	1.58
November- _b January ^b	0	ł	ł	ł	0	ł	ł	0	ł	ł
February	9	52.5	1.71	48-59	9	52.5	1.71	0	ł	ł
March	652	57.4	0.23	44-81	590	57.4	0.25	62	57.6	0.73
	1,630				1,358			272		

•

^bNone caught during November, December, or January.

^aData available only for 26-28 February.
	N	\overline{X} Length	SE	Range	Number of Recaptures
April	10	26.4	0.91	22-32	0
May	42	27.8	0.39	23-32	0
June	321	28.3	0.12	22-34	10
July	395	29.9	0.13	13-36	15
August	159	29.6	0.30	12-37	9
September	318	13.7	0.22	10-33	2
October	105	18.4	0.30	10-29	4
November	13	18.6	1.85	15-40	0
December	<u> </u>	16.0		16	0
	1,369				40

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Table 6. Numbers and body lengths (mm) of <u>Gastrophryne carolinensis</u> trapped in pitfalls along the periphery of Pond C Reservoir (1971). No specimens were caught February-March

	N	\overline{X} Length	SE	Range	Number of Recaptures
February ^a	0				
March	13	22.0	0.47	19-24	2
April	16	22.8	0.71	17-29	0
May	0				0
June	1	16.0		16	0
July	15	14.9	0.70	10-21	0
August	33	16.5	0.44	11-22	0
September	75	17.2	0.32	11-23	3
October	42	18.1	0.46	14-25	2
November	35	19.0	0.46	14-25	l
December	14	21.1	0.82	15-28	0
January	5	22.4	0.75	20-24	0
February	4	21.2	1.44	19-25	0
March	18	21.6	0.51	17-25	0
	271				8

Table 7. Numbers and body lengths (mm) of <u>Acris gryllus</u> trapped in pitfalls along the periphery of Pond C Reservoir (1971-1972)

^aData available only for 26-28 February.

The last species for which seasonal data are presented is <u>Pseudacris triseriata feriarum</u> (the upland chorus frog). Seven (4%) of the 173 specimens trapped and marked were recaptured (Table 8). Adult frogs were at the reservoir throughout the year and emergent transformees (7-11 mm) were caught in May and June.

Body lengths of <u>R</u>. <u>pipiens</u>, <u>B</u>. <u>terrestris</u>, and <u>G</u>. <u>carolinensis</u> caught in each trap area and each thermal zone were similar among total captures, initial captures and recaptures for each species (Tables 9, 10 and 11). Dispersal of the three species among traps, however, differs considerably. To allow for a comparison of trap affinities among the three dominant species, the numbers of specimens and trap rankings are presented together (Table 12). Although some trap sites were ranked consistently high for each species (A, B and C) and some consistently low (F, G and L), others exhibited considerable disparity among the three dominant species (V, W and Z). Data for <u>Acris gryllus</u> (Table 13) and <u>Pseudacris triseriata</u> (Table 14) both differ considerably among body lengths and numbers trapped.

In addition to determining which adult amphibians occur and breed in the vicinity of the heated reservoir, a major goal of the pitfall trapping study was to reveal the reservoir areas in which larval development occurred. Emergence patterns of immature <u>R</u>. <u>pipiens</u> along the periphery of Pond C Reservoir can be seen by comparing initial captures from each trap area (Table 15). Of 1,306 initially captured immature <u>R</u>. <u>pipiens</u>, 49% were caught in five (26%) of the 19 trap sites. All of these areas were associated with nearby cool seepage ponds or

	N	X Length	SE	Range	Number of Recaptures
February ^a	2	29.5		27-32	0
March	6	26.0	0.86	24-29	1
April	15	19.4	0.83	15-27	0
May	33	16.8	0.90	11-30	0
June	43	15.1	0.55	7-25	2
July	2	15.0		15	0
August	4	22.0	2.48	15-26	0
September	2	25.0		25	0
October	5	27.4	1.03	24-29	0
November	1	28.0		28	0
December	5	30.0	1.14	26-33	0
January	33	29.3	0.39	25-33	1
February	13	28.7	0.58	24-32	2
March	8	27.8	0.56	26-30	_1
	173				7

Table 8. Numbers and body lengths (mm) of <u>Pseudacris triseriata</u> trapped in pitfalls along the periphery of Pond C Reservoir (1971-1972)

^aData available only for 26-28 February.

of Pond (thermal (pond near	c Reserve gradient, r the tra	beginr beginr beginr beginr	nuy lengum n 26 Februi ning at the Fig. 2)	e cool er	to 31 M d. The	arch 1972. symbol (•	Trap.) indi	in picta s are gri cates th	puped bres	by zones a ence of a	long t cool s	i priery he eepage
		To	otal Captun	res		Initial C	apture	S		Recapti	ures	
Zone	Trap	z	X Length	SE	z	X Length	SE	Range	z	X Length	SE	Range
П	≮ຜ⊃ບ	188 178 69 99	38.2 32.9 37.2 37.2	0.66 0.48 0.96 0.77	169 133 85 85	38.0 33.3 37.4 36.9	0.69 0.61 0.99 0.78	25-67 21-59 25-68 28-62	14 14 14	39.9 31.7 34.2 38.7	1.27 0.59 4.04 2.74	30-62 26-50 25-42 27-60
	с >ш	69 221 108	33.1 34.9 33.6	0.63 0.43 0.57	54 183 89	33.3 35.3 33.5	0.76 0.48 0.65	25-59 22-60 23-68	15 38 19	32.4 32.7 34.4	0.98 0.97 1.10	27-43 22-54 29-50
III	L 3 0	25 171 25	33.3 42.0 32.2	0.78 0.77 0.82	23 118 22	33.2 41.2 32.6	0.75 0.95 0.89	28-40 22-75 27-42	33.2	35.0 43.6 29.3	 1.29 0.66	29-41 29-73 28-30
IV	нх₁	20 34 160	35.5 32.0 34.4	1.67 1.29 0.65	18 33 138	35.5 32.0 34.2	1.86 1.33 0.69	28-56 23-68 21-67	52-2	35.5 32.0 36.2	 1.82	35-36 32 27-56
>	╸≻⊻	136 84 55	34.5 31.8 34.7	0.57 0.64 0.84	106 77 46	34.3 31.9 34.2	0.65 0.67 0.86	20-54 20-57 25-57	30 9	35.3 31.0 37.5	1.20 2.18 2.58	27-60 21-40 31-56
N	M Z	40 87 10 10	38.5 28.5 33.4	1.36 0.94 2.70	36 79 10 1,484	37.5 28.2 33.4	1.28 0.93 2.70	23-61 20-56 26-55	4 8 295 295	47.2 31.1 	6.34 4.56 	30-60 23-61

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seepage	pond near	r the tr	ap									
		To	otal Captur	es		Initial C	apture	S		Recapti	ures	
Zone	Trap	z	X Length	SE	z	X Length	SE	Range	z	X Length	SE	Range
	• • •	262	46.7	1.15	227	46.0	1.31	6-83 5	35	51.5	1.17	33-68
Ι		435 15	53.U 46.0	00 3.10	303 14	53.2 45.2	0.58 3.22	19-58	2	57.0		
	ပ ပ	144	54.6	0.63	132	54.7	0.68	20-77	12	52.9	1.04	45-57
	۵	76	50.6	1.06	70	50.5	1.14	30-70	9	51.1	2.24	45-60
11	> 1	18	45.3	4.15	11	46.8	4.11	16-71		20.0		
	Ш	94	49.2	0.93	75	49.2	1.11	30-77	19	48.9	1.42	38-63
	Ŀ	45	50.1	1.43	33	50.3	1.83	20-71	12	49.5	1.95	37-57
III	3	40	50.3	1.42	31	48.9	1.69	26-60	ი	55.0	1.83	46-61
	G	36	48.0	1.63	32	47.7	1.82	32-71	4	50.7	0.85	49-53
1 1 1 1 1 1 1 1 1	H	44	48.1	1.61	37	47.2	1.84	20-73		52.8	2.36	41-62
IV	×	22	43.4	2.57	19	42.5	2.88	22-73	m	49.0	4.50	40-54
	• I	57	53.0	1.49	42	52.7	1.97	24-80	15	53.8	1.42	42-62
1 1 2 1 1 1 1 1	•0	88	52.0	0.79	99	52.3	1.01	20-72	22	51.1	0.94	44-58
>	۲	49	47.6	1.54	38	47.2	1.92	14-72	Ξ	49.1	1.82	36-56
	×	80	53.8	0.89	62	53.4	0.94	23-72	18	55.3	2.26	36-72
		30	50.1	1.32	23	50.0	1.53	35-62	2	50.1	2.85	41-62
١١	•	29	52.5	1.77	27	52.5	1.89	30-75	~	53.0		
	Σ	1,630	48.7	-	1, <u>358</u>	47.8	1.27	30-68	16 272	51.5	2.15	36-68

Table 10. Numbers and body lengths (mm) of <u>Bufo terrestris</u> captured in pitfall traps along the periphery of Pond C Reservoir from 26 February 1971 to 31 March 1972. Traps grouped by zones along the thermal gradient beginning at the cool and The sumbol (*) indicates the presence of a cool

the perification of the cool see	thermal sthermal age pond	gradie near t	keservoir int, beginn he trap	ing at t	he cool	end. The	symbo	cn 19/2.	licates	are grou the pres	ence o	zones Fa
		To	tal Captur	es.		Initial C	apture	S		Recapt	ures	
Zone	Trap	z	X Length	SE	z	X Length	SE	Range	z	X Length	SE	Range
ŀ	в. В.А.	127 109	27.6 27.7	0.41 0.33	122 107	27.5 27.7	0.43 0.34	13-36 16-35	ں م	28.8 28.5	0.80	26-31 28-29
1	5 0	76 134	27.5 25.9	0.50 0.53	75 133	27.5 26.0	0.50 0.53	12-34 13-35		33.0 18.0	11	::
	۵	43	29.3	0.40	42	29.3	0.41	19-36	-	29.0		1
II	> ш	85 49	25.2 28.5	0.71 0.62	83 46	25.0 28.2	0.72 0.63	12-34 16-36	2 N N	31.0 32.0	 2.64	27-35 28-37
	ш;	2	26.5		2	26.5		26-27	0	1		1
111	3 ()	43 27	25.6 29.1	0.68 0.68	43 27	25.b 29.1	0.54 0.68	17-33 16-34	00			
	н×	29 58	24.5	1.33 0.74	 26 54	24.5 27 0	1.45 0.79	10-33 14-40	- C 4	25.3 29.2	3.28	19-30
•	с н	36	27.1	1.00	35	24.4	0.98	14-36		16.0		3 }
1 1 1 1 1 1	J.	50	23.7	1.02	48	23.6	1.05	13-35	2	25.0	1 1 1 1 1 1 1	20-30
>	≻⊻	86 27	26.9 26.3	0.58 1.12	84 26	26.7 26.5	0.58 1.14	14-35 17-36	~~	31.5 20.0		27-36
		16	30.9	0.46	15	31.0	0.47	27-35	-	29.0	1	1
١٨	• ~ ¥	342 17	17.7 29.7	0.44 0.46	332 16	17.3 29.6	0.44 0.92	10-35 20-35	0	29.5 31.0	0.61 	27-34
		1,356			1,316				40			

Table 11. Numbers and body lengths (mm) of Gastrophryne carolinensis captured in pitfall traps along

Gastrophry 26 Februar symbol (•)	ne <u>caroline</u> y 1 <u>971 to 3</u> indicates	ensis (<u>G.c</u> 31 March 1 the prese	.) trappe 972. Tra nce of a (d in pitfa ps are gro cool seepa	lls along t uped by zon ge pond nea	he periphes along the trans	hery of l the then Ip	Pond C Res rmal gradi	ervoir from ent; the
		Z	per Speci	es	LatoT	Abund	lance Rar	nkings	
Zone	Trap	<u>R.</u> p.	<u>B.t</u> .	<u>е.</u> с.	N	R.P.	<u>B.t</u> .	<u> </u>	Average Rank
	Α.	188	262	127	577	2	2	r	-
⊢	• œ :	178	435	109	722	က်	-	4	2
4	- 0	66 66	15 144	76 134	160 377	11.5 8	<u>6</u> 8	~ ~	<u></u>
8 9 9 9 9 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	D		76	43	188	11.5	7	11.5	
11	>	221	18	85	324		18	9	6.5
	ш	108	94	49	251	7	4	10	2
 		25	45	2	72	16.5	11	19	18
III	3	171	40	43	254	4	13	11.5	10
	J	25	36	27	88	16.5	14	15.5	17
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	H H	20	44	29	93	18 	12	14	15.5
١٧	×	34	22	58	114	15	17	8	14
	•	160	57	36	253	2	ი	13	6
0 0 0 0 1 0 0 0 0 0	•0	136	88	50	274	9	5	6	4
>	7	84	49	86	219	10	10	Ω.	6.5
	⊻	22	80	27	162	13	و	15.5	12
		40	30	16	86	14	15	18	19
١٨	• 2	87	29	342	458 20	6,	16 1	-;	י י י
	Σ	01	000 1,630	1,356	$\frac{93}{4,765}$	<u>6</u>	x	2	د.دا د
		•	•	•	•				

Numbers of three species of anurans: Rana pipiens (R.p.), Bufo terrestris (B.t.) and Table 12.

Thermal Zone	Trap	N	X Length	SE	Range
	А•	17	16.9	0.62	21-21
	B●	18	19.2	0.71	14-24
Ι	U	16	16.2	0.47	14-20
	C	9	19.5	1.15	11-22
	D	9	21.6	0.74	19-26
II	V	3	18.6	1.45	16-21
	E	21	19.7	0.75	15-25
	F	5	22.2	1.02	19-25
III	W	22	18.5	0.67	13-25
	G	6	18.8	1.30	15-23
	н	5	16.6	1.36	15-22
IV	Х	1	17.0		17
	I•	60	18.1	0.48	11-28
	ј•	33	16.9	0.55	10-25
۷	Y	3	20.3	1.45	18-23
	К	14	18.2	0.88	14-23
	L	11	20.5	0.90	15-25
VI	Z•	2	21.0		17-25
	м	16	21.2	0.70	17-29
		271			

Table 13. Numbers and body lengths (mm) of <u>Acris gryllus</u> captured in pitfall traps along the periphery of Pond C Reservoir from 26 February 1971 to 31 March 1972. Eight specimens were recaptured. The symbol (*) indicates the presence of a cool seepage pond near the trap

Table 14. Numbers and body lengths (mm) of <u>Pseudacris triseriata</u> captured in pitfall traps along the periphery of Pond C Reservoir from 26 February 1971 to 31 March 1972. Seven specimens were recaptured. The symbol (*) indicates the presence of a cool seepage pond near the trap

Thermal Zone	Trap	N	\overline{X} Length	SE	Range
	A•	6	19.0	3.32	7-26
	B•	7	27.3	1.70	19-32
Ι	U	2	25.5		22-29
	D	3	28.0	1.52	26-31
	D	0			
II	V	13	20.2	1.61	15-30
	Ε	6	30.8	1.40	24-33
	 F]	30.0		30
III	W	3	21.7	3.66	18-29
	G	2	29.5		29-30
	н	1	32.0		32
IV	X	7	24.4	1.27	19-30
	I•	3	24.3	3.38	20-31
	 ј•	0			
۷	Y	9	24.3	1.52	16-32
	К	1	32.0		
	 L	2	30.0		28-32
VI	Z●	106	20.1	0.68	11-32
	м	0			
		172			

Table 15. Numbers of immature (body length <47 mm) Rana pipiens initially captured in pitfall traps. The symbol (+) indicates that the sample includes at least one emergent transformee (< 30 mm). An additional 31 specimens were trapped from November 1972 to March 1973. Traps are grouped by zones along the thermal gradient beginning at the cool end. The symbol (•) indicates the presence of cool seepage pond near trap

Thermal Zone	Trap	Feb ^a	Mar	Apr	May	Jun	յսլ	Aug	Sep	0ct	Total	Abundance Ranking	Transformees (No. of Months)
I	• ▼¤⊃∪	- 20 E	54 54 54	4054	21+ 58+ 21+ 11+	294 274 164 25	57 2 84 144	90 + €	*000	*000	133 116 54 73	1 3 11 7.5	ი ი 4 ო
II	<u>о</u> >ш	2 7 7	*- *	1 37 4 13	23+ 87+ 35+	94 204 11+	ტ.ფ.ჭ	-00	0-0	000	52 58 79	12 10 6	ৰ ল ৰ
III	L 3 G	а 9 – 1 С	ი – ი	40-	5 5 18 18	2 35 4	6 + 6	-40	020	0 m 0	22 80 22	16.5 5 16.5	-0-
IV	н×н	0 M B	3 14 14	4 0 + 0	1 7+ 26+	32 + 32	3 6 4 12 4	000	00*	00 +	14 32 123	18 14 2	0 7 30
>	•×	و 10-	3+ 14+ 5	30 9 9 9	21+ 7+ 7+	354 144 134	84 114 6	4 4 4	000	* 0 *	95 69 44	4 13 13	77
١٨	r M €	-00	2 2 +	4 4 0	-1 8 6	39 + 39 +	6 17+ 2+	005	000	000	29 73 7	15 7.5 19	- 22

^aData available only for 26-28 February.

cool reservoir shallows. Traps associated with dry wooded areas generally caught the fewest numbers of <u>R</u>. <u>pipiens</u>. Of the immatures, 73% occurred in nine (47%) of the trap sites; none of which were surrounded by woody habitat.

Of 230 initially captured <u>B</u>. <u>terrestris</u>, 50% occurred in four (21%) of the pitfall trap sites. Of the initially captured immatures, 74% occurred in eight (42%) of the trap areas. Unlike the more aquatic <u>R</u>. <u>pipiens</u>, the terrestrial toads were encountered more commonly in trap areas near woods and were less restricted to sites near cool ponds or reservoir seepage.

High numbers of initially trapped immature <u>R</u>. <u>pipiens</u> (<30 mm) usually represented some emergent transformees (Table 15). Although transformed young were encountered other months of the year (except February 1973), only 35 (2.7%) were trapped from November to March. The monthly occurrence of emergent young was not greatest in traps with the highest total capture. When the 19 trap areas are ranked according to total catch of initially captured immature <u>R</u>. <u>pipiens</u>, 49% of the specimens are found to occur in five (26%) of the traps, and 90% of the specimens occurred in 13 (68%) of the traps. The temperature of Pond C Reservoir during the breeding season undoubtedly determines whether or not larvae can develop successfully. As indicated by Table 15, emergent transformees were trapped near pitfall site Y (Figs. 2 and 12) from February to July 1972. No transforming larvae were trapped there in January, February or March of the next year, however.

Fig. 12. Pitfall-drift fence section Y (Fig. 2). Partial separation of the seepage basin by land may keep water temperature at sub-lethal levels. Emergent young of <u>Rana pipiens</u> were trapped here from February to August 1971.



Figure 12

A grouping of data into the six major temperature regimes along the thermal gradient (Table 1, Fig. 2) shows that the numbers of <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> trapped generally decreased at the heated extreme (Tables 16 and 17). Numbers of total captures, initial captures, and initially captured immatures all demonstrate the same phenomenon in generally conforming to the thermal gradient along the reservoir's cool arm. <u>Gastrophryne carolinensis</u>, <u>A</u>. <u>gryllus</u> and <u>P</u>. <u>triseriata</u>, however, do not clearly reflect this gradient in the numbers trapped (Tables 18 and 19).

Thermal Zone	Total Captures (N = 1,779)	Initial Captures (N = 1,484)	Innitial Captures (Immatures) (N = 1,306)	Recaptures (N = 295)
I	133.5	113.0	95.5	20.5
II	132.7	108.7	96.7	24.0
III	73.7	54.3	42.0	19.3
IV	71.3	63.0	57.3	8.3
v	91.7	76.3	71.3	15.3
VI	45.7	41.7	37.7	4.0

Table 16. Mean numbers of <u>Rana pipiens</u> caught per pitfall trap area in each of the thermal zones along the periphery of Pond C Reservoir

Thermal Zone	Total Captures (N = 1,636)	Initial Captures (N = 1,364)	Initial Captures (Immatures) (N=230)	Recaptures (N = 272)
I	214.8	184.0	22.0	30.0
II	62.7	54.0	14.0	8.7
III	40.3	32.0	9.0	8.3
IV	41.0	32.7	9.3	8.3
v	72.3	55.3	7.0	17.0
VI	42.3	33.3	8.0	8.3

Table 17. Mean numbers of <u>Bufo</u> terrestris caught per pitfall trap area in each of the thermal zones along the periphery of Pond C Reservoir

Table 18. Mean numbers of <u>Gastrophyne carolinensis</u> caught per pitfall trap area in each of the thermal zones along the periphery of Pond C Reservoir

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Thermal Zone	Total Captures (N = 1,356)	Initial Captures (N = 1,316)	Recaptures (N=40)
I	111.5	109.2	2.2
II	59.0	57.0	2.0
III	24.0	24.0	0
IV	41.0	38.3	2.7
V	54.3	52.7	1.7
VI	125.0	121.0	4.0

Thermal Zone	<u>A. gryllus</u> (N = 272)a	<u>P</u> . <u>triseriata</u> (N = 173) ^D
I	15.0	4.5
II	11.0	6.3
III	11.0	2.0
IV	22.0	3.7
٧	16.7	3.3
VI	· 9.7	36.0

Table 19. Mean numbers of <u>Acris gryllus</u> and <u>Pseudacris</u> <u>triseriata</u> caught per pitfall trap area in each of the thermal zones along the periphery of Pond C Reservoir

^aEight recaptures.

^bSeven recaptures.

Not only were <u>R</u>. <u>pipiens</u> trapped every month of the year at Pond C Reservoir (Table 3), but young frogs emerged for 11 months of the year there, compared with two or three months elsewhere on the S. R. P. at Risher Pond (1969-1970) and Karen's Pond (1970). Extended breeding at Pond C Reservoir was not accompanied by an apparent size differential among transformed young (Table 20).

<u>Bufo terrestris</u> at Pond C Reservoir demonstrated a seasonal activity pattern similar to that at Karen's Pond in 1970 (Table 21). Most larvae from Pond C Reservoir emerged at about the same time of year as they did at Karen's Pond (1970), but may have emerged at a smaller size (cf. Tables 20 and 21). No transformed <u>R</u>. <u>pipiens</u> emigrated from Risher Pond either year.

Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along the periphery of three aqua habitats on the Savannah River Plant in South Carolina. Numbers in parentheses indicate percentage of total captures taken during period indicated	tic	the	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along the periphery of three habitats on the Savannah River Plant in South Carolina. Numbers in parentheses indic percentage of total captures taken during period indicated	aqua	ate	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along the periphery of the habitats on the Savannah River Plant in South Carolina. Numbers in parentheses is percentage of total captures taken during period indicated	nee	indic	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along the periphery on the Savannah River Plant in South Carolina. Numbers in parenthem percentage of total captures taken during period indicated	oft	ses	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along the periph habitats on the Savannah River Plant in South Carolina. Numbers in pare percentage of total captures taken during period indicated	ery	nthe	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along the pe habitats on the Savannah River Plant in South Carolina. Numbers in percentage of total captures taken during period indicated	riph	pare	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps along th habitats on the Savannah River Plant in South Carolina. Numbers percentage of total captures taken during period indicated	e pe	in	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps alon habitats on the Savannah River Plant in South Carolina. Num percentage of total captures taken during period indicated	g th	bers	
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall traps habitats on the Savannah River Plant in South Carolina. percentage of total captures taken during period indicat	alon	Mun	ed
Table 20. <u>Rana pipiens</u> trapped yearly in pitfall tr habitats on the Savannah River Plant in South Caroli percentage of total captures taken during period inc	aps	na.	licat
Table 20. <u>Rana pipiens</u> trapped yearly in pitfal habitats on the Savannah River Plant in South Ca percentage of total captures taken during period	l tr	r Io	inc
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Table 20. <u>Rana pipiens</u> trapped yearly i habitats on the Savannah River Plant in percentage of total captures taken durin	n pi	Sout	g pe
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Table 20. <u>Rana pipiens</u> trapped habitats on the Savannah River P percentage of total captures tak	year	lant	en d
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Table 20. <u>Rana pipiens</u> habitats on the Savannah percentage of total capt	trapl	Riv	ures
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Table 20 habitats percenta	ا∽ ∙	u	ge o
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		Jaco 20 oddaroW	Months when Emergent	Range of Body	Speci	mens < Body Lei	26 mm ngth
Area	Z	MONUTS OF FEAK Activity	roung were Captured	(mm)	z	X	SE
Karen's Pond ^a 1970	183	March (17%) June-July (50%)	June-July	23-71	15	26.5	0.52
Risher Pond ^a 1969	984	February-April (78%) July (8%)	June-August	23-88	48	26.6	0.25
Risher Pond ^a 1970	866	February-March (50%) June-July (26%)	June-August	22-86	61	27.5	0.20
Pond C Reservoir 1971	1,699	May-July (66%)	all months except November & February	20-75	302	26.5	0.13

^aData from J. W. Gibbons (1974, in press and in preparation).

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pitfalls al	Carolina.	period ind
yearly in p	nt in South	during the
trapped	iver Pla	es taken
errestris	avannah R	al capture
Bufo te	on the Si	e of toti
Table 21.	habitats	percentag

		Months of Dock	Months when Emergent	Range of Body	Speci	imens < Body Le	26 mm ngth
Area	z	Activity	captured	(mm)	z	×	SE
Karen's Pond ^a 1970	1,784	March-May (55%) July-August (36%)	June-August	7-80	34	10.9	0.14
Risher Pond ^a 1969	277	April-May (79%)	none	33-76	0	:	:
Risher Pond ^a 1970	572	March-April (79%)	none	25-69	0	:	!
Pond C Reservoir 1971	968	March-April (52%) June (35%)	May-July	5-81	31	10.8	0.15

^aData from J. W. Gibbons (1974, in press and in preparation).

Field Study: Reservoir Sampling with Dipnets and Minnow Traps

Nine species of amphibians (seven anurans and two urodeles) were removed from aquatic habitats during the 15 month sampling period (Table 22). Very few salamanders were encountered in heated areas of the reservoir. Two larval <u>Manculus quadridigitatus</u> (dwarf salamander) and only one adult <u>Siren intermedia</u> (lesser siren) were removed from a heated area (V). Four anuran species occurred in all areas sampled to some extent. Species diversity was highest in a cool pond not contiguous with the reservoir (cool pond Z) and lowest in the hotter reservoir location, area K (Figs. 2 and 5-8; Table 1).

Larval amphibians of only one species (<u>Rana pipiens</u>) were encountered in numbers high enough to allow for a comparison among areas. Data from routine monthly dipnet samples and from the minnow trapping survey were pooled to compare growth among thermally distinct areas (Fig. 13). Plotting of the data for all cool ponds showed that they were virtually the same. Data from Pond Z were chosen to represent growth at specific stages of development in a cool (control) habitat. Reservoir areas V and K were both heated; data from the two areas are shown to be smaller at virtually every stage of development than larvae at cool Pond Z (Fig. 13).

lable 22. Larval a 1971 to September 1	mphibians present (+) 972) in the vicinity o	or absent (0) f Pond C Reser	in one or more voir (^{&} = eggs	of 15 monthly deposited but	dıpnet sam no larvae f	ples (June ound)
Species	Cool Pond Z, Not Contiguous with Reservoir	Cool Pond A, Contiguous with Reservoir	Cool Pond I, Contiguous with Reservoir	Cool Pond B, Contiguous with Reservoir	Area V, Shallows of Reservoir	Area K, Seepage into Reservoir
Rana pipiens	+	÷	÷	+	+	+
Bufo terrestris	+	+	+	+	+	+
Acris gryllus	+	+	+	+	+	+
<u>Hyla</u> cinerea	+	+	+	+	+	+
<u>Gastrophryne</u> <u>carolinensis</u>	+	+	0	+	+	+
Rana clamitans	+	+	+	+	0	0
<u>Notophthalmus</u> viridescens	+	+	+	0	0	0
<u>Manculus</u> <u>quadridigitatus</u>	+	0	0	0	+	0
<u>Hyla versicolor</u>	+	0	Ο	0	0	0
Total no. species X no. species/month	9 3.1	2.4	6.1	6 1.6	9].]	4& 0.6
% samples with no larvae	ο	0	O	14.3	14.3	57.1

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Fig. 13. Total lengths of larval <u>Rana pipiens</u> collected from three reservoir sites receiving differential levels of thermal loading. Pond Z (Fig. 2) is a cool seepage area unaffected by thermal effluent. Shore V (Fig. 7) and Shore K (Fig. 8) are two sites in the heated reservoir where larval development occurred. Few specimens were collected from the hottest sampling station (K). Lengths are compared at specific stages of development (Gosner, 1960).

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<u>Field Study: Reservoir Sampling of Larval</u> <u>Bufo terrestris</u>

Larval B. terrestris were sampled weekly at five locations in the vicinity of Pond C Reservoir (Fig. 9). The majority of specimens examined in this study is assumed to be the result of eqg masses deposited in the study area on or just prior to 22 March (Table 23). A consistent progression of advancing stages and increasing sizes was observed weekly in all areas except area K (Table 24; Fig. 14). Larvae were more advanced and larger in heated water than cool Pond B. Data from the supplemental analysis only (n = 20) were used for statistical tests. Larvae from area K, however, consisted of data from the initial analysis (n = 10). After the first sampling period, total lengths and stages of development were positively related to the level of thermal loading in all areas except K (Table 24). Following the breeding migration, developing larvae and three separate egg masses were found at area K, but more than 95% of the eggs were dead. Fewer than 20 living embryos were found among the three egg masses present; all of these were located in the clutch closest to shoreline seepage. Several toad larvae, however, were removed from area K in the small (n = 10)samples of 29 March and 5 April.

Stages of development and lengths of larvae taken in weekly samples reveal a reversal from the initial relationship among the three areas. Statistical analysis of successive samples indicates that growth and development were significantly different among areas in the first and third weekly sample (Table 25). At a given stage, however, larvae developing in cool water were larger than those reared in heated areas (Table 26).

Date (1972))	Rainfall (cm)	No.	Trapped
March	2	0		0
	4	0.10		2
	8	0.15		0
1	1	0		0
1	15	0.05		0
1	8	2.26		30
2	22	2.31		562
2	25	0		11
2	27	0		0
2	29	1.47		5
April	1	1.22		31

Table 23. Adult <u>Bufo terrestris</u> trapped in pitfalls along Pond C Reservoir. Traps were checked at three day intervals or after precipitation during the preceding night

Cool Pond BArea AArea VArea VArea D $24.4 C$ $24.4 C$ $32.9 C$ $34.4 C$ $34.9 C$ $35.7 C$ Temperature $\overline{X} \pm SE$ $3-29-72$ 15.7 ± 0.42 14.8 ± 0.25 14.0 ± 0.31 area not 14.4 ± 0.12 31.7 ± 0.22 31.5 ± 0.17 30.8 ± 0.25 14.0 ± 0.31 area not 14.4 ± 0.12 $5tage$ 31.7 ± 0.22 31.5 ± 0.17 30.8 ± 0.25 34.2 ± 0.63 30.3 ± 0.12 $5tage$ 31.7 ± 0.22 31.5 ± 0.17 30.8 ± 0.25 34.2 ± 0.63 30.2 ± 0.51 $10 tal length$ 16.6 ± 0.67 17.2 ± 0.65 34.2 ± 0.63 39.8 ± 0.51 11.6 ± 0.12 $4-5-72$ 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae $nond$ $4-12-72$ Total length 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae $nond$ $4-12-72$ Total length 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae $nond$ $4-19-72$ Total length 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae $nond$ $4-19-72$ Total length 19.2 ± 0.38 $nostined$ no $narvaenondnond4-19-72190236.2 \pm 0.7034.2 \pm 0.6937.6 \pm 0.70nondnondnond4-19-721000030.7 \pm 0.6937.6 \pm 0.7010000100001000010000<$				Study Areas ^a		
329-72 31.7 ± 0.42 14.8 ± 0.25 14.0 ± 0.31 area not 14.4 ± 0. 3-29-72 15.7 ± 0.42 14.8 ± 0.25 14.0 ± 0.31 area not 14.4 ± 0. 5 tage 31.7 ± 0.22 31.5 ± 0.17 30.8 ± 0.25 sampled 30.3 ± 0. 5 tage 31.7 ± 0.22 31.5 ± 0.17 30.8 ± 0.25 sampled 30.3 ± 0. 4-5-72 17.2 ± 0.64 18.2 ± 0.66 22.8 ± 0.51 11.6 ± 0. 4-5-72 17.2 ± 0.65 34.2 ± 0.66 22.8 ± 0.51 11.6 ± 0. 5 tage 32.1 ± 0.52 33.5 ± 0.65 34.2 ± 0.66 22.8 ± 0.51 11.6 ± 0. 4-12-72 10 ength 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larvae 4-12-72 190th 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larvae 5 tage 32.7 ± 0.54 34.2 ± 0.69 37.6 ± 0.70 found found found 4-19-72 19.2 ± 0.38 insufficient no larvae no larvae no larvae 5 tage 36.2 ± 0.70 sample size found found <t< th=""><th>Temperatureb</th><th>Cool Pond B 24.4 C <u>Y</u> + SF</th><th>Area A 32.9 C <u>Y</u> + cF</th><th>Area V 34.4 C <u>V</u> + SF</th><th>Area D 34.9 C <u>Y</u> + SF</th><th>Area K 35.7 C <u>¥</u> + cF</th></t<>	Temperatureb	Cool Pond B 24.4 C <u>Y</u> + SF	Area A 32.9 C <u>Y</u> + cF	Area V 34.4 C <u>V</u> + SF	Area D 34.9 C <u>Y</u> + SF	Area K 35.7 C <u>¥</u> + cF
Stage 31.7 ± 0.22 31.5 ± 0.17 30.8 ± 0.25 sampled 30.3 ± 0.12 $4-5-72$ 17.2 ± 0.67 17.2 ± 0.64 18.2 ± 0.66 22.8 ± 0.51 11.6 ± 0.12 Total length 16.6 ± 0.67 17.2 ± 0.65 34.2 ± 0.63 39.8 ± 0.30 26.9 ± 0.12 $4-12-72$ 32.1 ± 0.52 33.5 ± 0.65 34.2 ± 0.63 39.8 ± 0.30 26.9 ± 0.12 $4-12-72$ 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larvae $4-12-72$ 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larvae $4-12-72$ 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larvae $4-12-72$ 16.8 ± 0.40 17.5 ± 0.52 20.10 ± 0.68 no larvae no larvae $4-12-72$ 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larvae $4-19-72$ 10.70 32.7 ± 0.54 34.2 ± 0.69 37.6 ± 0.70 60.00 $4-19-72$ 19.2 ± 0.38 $insufficientno larvaeno larvae4-19-7219.2 \pm 0.38insufficientno larvaeno larvae36.2 \pm 0.70sample sizefoundfoundfounda^{d} columned R vaccived no heated effluent: areas A V D and K vaccive foundfound$	3-29-72 Total length	15.7 ± 0.42	14.8±0.25	14.0±0.31	area not	7 = 35 14.4 ± 0.64
Total length 16.6 ± 0.67 17.2 ± 0.64 18.2 ± 0.66 22.8 ± 0.51 11.6 ± 0.15 Stage 32.1 ± 0.52 33.5 ± 0.65 34.2 ± 0.63 39.8 ± 0.30 26.9 ± 0.25 4-12-72 Total length 16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvae no larva Stage 32.7 ± 0.54 34.2 ± 0.69 37.6 ± 0.70 found found found found found found found $4-19-72$ Total length 19.2 ± 0.38 insufficient no larvae no larvae no larva found found found found 36.2 ± 0.70 sample size found found found found found found 36.2 ± 0.70 found fou	Stage 4-5-72	31.7±0.22	31.5±0.17	30.8 ± 0.25	sampled	30.3 ± 0.52
Total length16.8 ± 0.40 17.5 ± 0.52 20.1 ± 0.68 no larvaeno larvaeno larvaeStage32.7 ± 0.54 34.2 ± 0.69 37.6 ± 0.70 foundfound4-19-724-19-7219.2 ± 0.38 insufficientno larvaeno larvaeno larvaStage36.2 ± 0.70 sample sizefoundfoundfoundfound	Total length Stage 4-12-72	16.6 ± 0.67 32.1 ± 0.52	17.2 ± 0.64 33.5 ± 0.65	18.2 ± 0.66 34.2 ± 0.63	22.8±0.51 39.8±0.30	11.6±0.30 26.9±0.18
Total length 19.2±0.38 insufficient no larvae no larvae no larva Stage 36.2±0.70 sample size found found found found ^a rool nond R were in the heated	Total length Stage <u>A-</u> 10-72	16.8 ± 0.40 32.7 ± 0.54	17.5±0.52 34.2±0.69	20.1 ± 0.68 37.6 ± 0.70	no larvae found	no larvae found
	Total length Stage ^a Cool	19.2 ± 0.38 36.2 ± 0.70 nond R mereived no	insufficient sample size heated effluent:	no larvae found	no larvae found and K were in the	no larvae found

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reservoir proper.

^bMean of maximum temperatures recorded on maximum-minimum thermometers over periods of 2-3 days during March 1972 (n = 15).

Fig. 14. Mean stage of development (Limbaugh and Volpe, 1957) of larval <u>B</u>. <u>terrestris</u> collected in three weekly samples from three areas along Pond C Reservoir. Vertical lines represent ± 2 SE.



		St	age of Develo	opment	
	X St	age by Study	Area		
	В	Α	V	Kruskal-W	allis Anova
Date	24.4 C	32.9 C	34.4 C	Н	Р
3-29-72	31.7	31.5	30.8	6.06*	< 0.05
4-5-72	32.1	33.5	34.2	4.53	< 0.50
4-12-72	32.7	34.2	37.6	13.39*	< 0.005
			Total Lengt	:h	
	X Total	Length by St	udy Area	Kining he 7 hl	allia Amaua
	В	A	<u>v</u>	Kruska I-W	allis Anova
Date	24.4 C	32.9 C	34.4 C	Н	Р
3-29-72	15.7	14.8	14.0	7.46*	< 0.025
4-5-72	16.6	17.2	18.2	2.48	< 0.50
4-12-72	16.8	17.5	20.1	12.74*	< 0.005

Table 25. Comparisons of stages of development and total lengths (mm) of larval toads collected in weekly samples from three thermal regimes (n = 20)

Table 26. Mean total lengths (mm) of larval toads at comparable stages of development, collected from cool and heated areas; (n) = sample size. Data for heated areas A and V are pooled for anova comparison with cool area B

••••••••••••••••••••••••••••••••••••••		Study Areas		Kruskal-Wall Anova		
Stage	B24.4 C	A32.9 C	V34.4 C	Н	Р	
31	14.7 (20)	14.2 (21)	14.2 (16)	4.11*	< 0.05	
32	16.0 (16)	15.5 (14)	15.8 (12)	0.44	< 0.50	
33	18.0 (24)	16.9 (16)	16.7 (6)	5.04*	< 0.025	

*Statistically significant.

Laboratory Study: Rearing of Larval Rana pipiens

Larval development through metamorphosis occurred at all experimental temperatures except 35 C (Table 27). All embryos maintained at that temperature died within nine days. Hence, data from this temperature will be excluded in comparisons of the thermal regimes. Of the 1.200 specimens introduced at the three other temperatures, 57%transformed into late metamorphic stages (41-46). Accidental deaths represented less than 5%, and mortality accounted for 30% of the original experimental animals. Data were not available for approximately 9% of the 1,200. Mortality was greatest at 30 C, and transformation greatest at 25 C. An expression of comparable mortality (survivorship) can be made by using a ratio of the two parameters. Values of the transformation rates/mortality rates are 1.81 at 20 C, 2.25 at 25 C and 1.72 at 30 C. These figures suggest that the 25 C regime was most favorable and 30 C regime least favorable to larval development. Approximately 70% of the larvae at the three temperatures transformed; approximately 30% were lost due to mortality (Table 28).

Marked differences occurred in both transformation rates and sizes at the three temperatures having normal development. Completion of development by the larvae required more than five (30 C), seven (25 C) and 17 (20 C) months (Fig. 15). Developmental rates were positively realted to temperatures, and transformation apparently required an inordinate amount of time at 20 C. Numbers of specimens transforming were comparable among replicate aquaria at the same temperatures (Table 29), and did not differ significantly from one

Temper- atures	Replicate Aquaria	Accidental Deaths	Mortality	Transfor- mations	Specimens Not Accounted For
	А	3	29	47	21
20 C	В	2	23	49	26
20 0	С	10	27	51	12
	D			56	
		4.8%	28.0%	50.8%	16.5%
	A	1	27	64	8
	В	13	39	43	5
25 C	С	2	28	67	3
	D	0	17		7
		4.0%	27.8%	62.5%	5.8%
	Α	7	44	47	2
	В	3	32	60	5
30 C	С	6	30	63	1
	D	5	28	60	
		5.2%	33.5%	57.5%	3.8%
	A	0	92	0	8
	В	2	70	0	28
35 C	С	0	78	0	22
	D		73		
		58	670	683	187 ^a

Table 27. Laboratory reared <u>Rana pipiens</u>; 100 embryos (stage 11 or 18) originally placed in each aquarium. Percentages are based on a total of 400 specimens at each temperature

^aThree transformed specimens escaped.

Temperature	N	Mortality	Transformation	
20 C	315	36%	64%	
25 C	361	30%	70%	
30 C	364	37%	63%	

Table 28. Comparison of larval mortality and transformation at three thermal regimes (excluding specimens not accounted for)

Table 29. Body lengths (mm) of 683 transforming <u>Rana pipiens</u> reared at three different temperatures in the laboratory

Aquarium	N	X	SE	Range	CV
20A	47	28.6	0.45	22-35	10.8
20B .	49	29.3	0.48	22-36	11.4
20C	51	30.8	0.48	23-40	11.2
20D	56	31.3	0.46	24-40	10.9
25A	64	26.6	0.29	22-34	8.7
25B	43	25.5	0.39	19-32	9.9
25C	67	26.0	0.28	20-32	8.8
25D	76	27.0	0.26	23-32	8.4
30A	47	24.7	0.40	20-33	11.0
30B	60	24.2	0.34	20-30	10.8
30C	63	24.6	0.32	20-30	10.3
30D	60	23.6	0.35	15-29	11.3
	683				

Fig. 15. Mean body lengths (\pm 1 SE) of laboratory reared larval <u>R. pipiens</u> transforming monthly at different thermal regimes; 400 embryos (stage 11 or 18) were originally introduced at each temperature. An asterisk indicates that the series was terminated (20 C and 30 C) before all larvae had transformed.


another $(X^2 = 18.6, P > 0.05)$. Body lengths of transformed larvae among replicate aquaria were significantly different at 20 C and 25 C but not at 30 C (Table 30). Differences in body lengths of transformees reared at the three temperatures were also highly significant (Table 30). Final transformees at each temperature were larger in size than larvae developing earlier (Table 31).

Biweekly transformation curves (survivorship excluding "natural" mortality) of larvae were similar for those reared at 25 C and 30 C. The transformation rate at 20 C, however, differed considerably from the other temperatures (Fig. 16). The transformation curves for the 25 C and 30 C series resemble a geometric progression, and the curve for larvae maintained at 20 C more closely approximates an arithmetic (additive) progression, being more linear in appearance. Larval survivorship (Fig. 17) was calculated by adding "natural" mortality and transformation. The proportions of the experimental populations remaining in each thermal regime over time, therefore, follow trends similar to those of the transformation curves (survivorship excluding mortality). Larvae reared at 25 C and 30 C demonstrated rapid declines, whereas those at 20 C declined gradually. Since the series at 20 C and 30 C were both terminated, data were plotted with the assumption that all of those living at the time would eventually complete metamorphosis.

Extrapolating data from the survivorship curves (Fig. 17), Zero survivorship should have occurred after 24 weeks at 30 C, 28 weeks at 25 C and 88 weeks at 20 C. The amount of time required to reach specified levels of survivorship are compared among the three

Temperature	N	x	Kruskal-Wallis Statistic (H)	Р
	47	28.6	17.02*	0.005
	49	29.3		
20 C	51	30.8		
	56	31.3		
	64	26.6	10.06*	0.025
	43	25.5		
25 C	67	26.0		
	76	27.0		
	47	24.7	2.78	0.05
	60	24.2		
30 C	63	24.6		
	60	23.6		
20 C	203	30.1	277.57**	0.001
25 C	250	26.4		
30 C	230	24.2		

Table 30. Comparisons of body lengths of <u>Rana pipiens</u> reared in the laboratory at three different temperatures. (Kruskal-Wallis nonparametric analysis of variance)

*Statistically significant.

Temper- ature	Month	N	X	SE	Range	C۷
30 C	October, 1971 November December January, 1972 February	4 133 70 13 <u>10</u> 250	19.2 24.1 24.2 25.5 26.5	2.17 0.20 0.35 0.59 0.78	15-23 19-33 16-30 22-29 23-30	22.6 9.4 12.2 8.4 9.3
25 C	November, 1971 December January, 1972 February March April	78 95 40 25 10 <u>2</u> 250	26.5 27.1 26.8 25.7 25.4 29.5	0.21 0.24 0.42 0.42 0.82 2.50	20-32 19-32 23-34 23-32 20-28 27-32	7.1 8.7 9.9 8.1 10.2 12.0
20 C	January, 1972 February March April May June July August September October November December January, 1973 February	9 34 33 10 17 27 16 9 5 7 4 4 7 21 203	30.3 30.7 29.2 29.9 28.9 28.7 28.3 27.7 28.8 29.7 30.0 29.8 33.7 34.8	1.08 0.37 0.38 0.74 0.69 0.64 0.78 1.62 1.77 1.04 1.92 2.14 1.06 0.77	25-36 26-35 24-33 27-35 25-33 23-38 23-35 22-37 22-32 26-33 25-35 30-38 28-40	10.7 7.1 7.4 9.9 11.5 11.0 17.6 13.8 9.3 12.8 14.4 8.3 10.2

Table 31. Body lengths (mm) of 683 transforming <u>Rana pipiens</u> at three different temperatures in the laboratory (1971-1972). Specimens were introduced into aquaria on 1 September 1971

Fig. 16. Biweekly survivorship rates, excluding mortality, of laboratory reared larval <u>R</u>. <u>pipiens</u> at three different thermal regimes. The double line at 20 C and 30 C indicates that these series were terminated before all specimens had transformed.



Fig. 17. Biweekly survivorship, including mortality, of laboratory reared larval <u>R</u>. <u>pipiens</u> at three different thermal regimes. An asterisk indicates that the series was terminated before all larvae had transformed.



thermal regimes (Table 32). Although the time required to progress from one level to the next is quite similar at 25 C and 30 C, corresponding time intervals at 20 C are not comparable.

To compare overall survival in a constant time reference, percent survival was plotted as a percent of the total time required for each group to terminate (Fig. 18). Total time represents the actual duration of experiments. Since all embryos at 35 C died within nine days, the curve presented represents mortality (rather than survivorship), and must be cautiously related to the three other groups in which larvae did develop through transformation. After 20% of the total time had passed, all groups (except 35 C) dropped rather abruptly in survival. The actual time intervals represented are quite different in duration, varying from nine days (35 C) to more than 17 months (20 C). Corrected to a uniform time comparison, survivorship curves for larvae reared at 20 C, 25 C and 30 C closely resemble one another.

Table 32. Time required for laboratory reared larval <u>R</u>. <u>pipiens</u> to reach comparable levels of survivorship (transformees + "natural" mortality) at different thermal regimes. Numbers in parentheses indicate the number of weeks between successive survivorship intervals

Deursent		Weeks Required				
Survivorship	20 C	25 C	30 C			
90	11	10	7			
75	22 (11)	11 (1)	8 (1)			
50	30 (8)	14 (3)	11 (3)			
25	44 (14)	16 (2)	13 (2)			
10	66 (22)	20 (4)	16 (3)			

Fig. 18. Percent survivorship of <u>Rana pipiens</u> laboratory reared at three different thermal regimes. Survivorship (excluding accidental deaths) is expressed as a percentage of the total time required for development or death. All larvae at 35 C died within nine days; thus, data for this group represent mortality only.



Comparison of body lengths at the later stages of development among the experimental thermal regimes demonstrates that size is negatively related to temperature (Fig. 19). Animals reared at warmer temperatures were smaller almost without exception, at any given stage of the final eight metamorphic stages (Table 33). Subjected to the Kruskal-Wallis nonparametric analysis of variance, differences in body lengths are shown to be statistically significant among the three thermal regimes (stage 42: H = 33.7, P < 0.005; stage 43: H = 50.1, P < 0.005; stage 44: H = 155.6, P << 0.005).

Two major abnormalities encountered among the three thermal regimes were the manifestation of crooked spines (Fig. 20, vertebral columns bent just behind the pelvic girdle) and paralyzed hindlimbs. Larvae with edemaceous and emaciated body conditions (Fig. 21) were also observed. The occurrence of larvae with crooked spines, as well as paralyzed hindlimbs, was significantly greater in aquaria at the higher thermal regimes (Table 34). Overall data suggest that larvae with crooked spines were shorter than those with normal spines (Fig. 22, Table 35). The numbers of specimens affected by crooked spine in each thermal regimes are significantly nonrandom ($X^2 = 138.4$, P << 0.005). Comparison, however, (Table 36) shows that the mean body lengths at the three temperatures did not differ uniformly with regards to spine condition. Crooked spine abnormalities did not greatly affect body size at any of the temperature regimes (20 C: H = 1.2, P > 0.05; 25 C: H = 0.01, P > 0.05; 30 C: H = 5.74, P < 0.025).

Fig. 19. Mean body lengths (± 2 SE) of laboratory reared <u>R</u>. <u>pipiens</u> at three different thermal regimes. Larval lengths are compared at specific stages of development (Gosner, 1960).



Figure 19

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Table 33.	different

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3		20 C			25 C			30 C	
orage or Development	z	X	SE	z	X	SE	z	X	SE
39	4	32.2	2.92	0	ł	1	2	26.0	4.00
40	S	35.0	3.13	0	ł	!	-	26.0	;
41	27	31.6	06.0	2	23.5	0.50	2	28.0	2.00
42	31	29.2	0.52	16	24.8	0.64	35	24.1	0.55
43	29	29.3	0.48	26	26.2	0.41	29	23.4	0.34
44	103	29.8	0.28	175	26.8	0.17	137	24.6	0.20
45	2	29.0	2.00	29	25.9	0.33	21	23.9	0.56
46	0	ł	:	-	20.0	8 1	2	15.5	0.50
	201			241			229		

Fig. 20. Larvae (reared at 30 C) demonstrating abnormally developed "crooked spines." Some specimens are upside-down due to helical swimming patterns.





Fig. 21. Larval <u>R</u>. <u>pipiens</u> demonstrating edemaceous and emaciated body condition. Specimens demonstrating these anomalous body conditions were most common in the cohort reared at 20 C.



Figure 21

		Crooked Spine			
	20 C	25 C	30 C		
n	203	250	230		
% affected	21%	10%	74%		
obs. f	43	34	170		
exp. f	73.4	90.4	83.2		
	X ² = 138.3**	X ² = 138.3** (P << 0			
	Pa	ralyzed Hindlimb			
	20 C	25 C	30 C		
n	203	250	230		
% affected	۱ <i>%</i>	2%	4%		
obs. f	2	5	10		
exp. f	5.0	6.2 5			
	X ² = 7.5*	(p < (0.05)		

Table 34. Occurrence of developmental abnormalities in larval <u>Rana</u> <u>pipiens</u> laboratory reared at three different thermal regimes

*Statistically significant.

Fig. 22. Mean body lengths (± 2 SE) of laboratory reared larval <u>R</u>. <u>pipiens</u> having normal or crooked spines at three different thermal regimes.



Group)	N	X	SE	Range	CV
20 C	Normal spines	160	30.2	0.28	22-40	11.78
20 C	Crooked spines	43	29.5	0.49	24-38	10 .97
25 C	Normal spines	216	26.4	0.16	19-34	9.05
25 C	Crooked spines	34	26.4	0.37	23-32	8.19
30 C	Normal spines	60	25.1	0.29	21-30	9.05
30 C	Crooked spines	<u>170</u> 683	23.9	0.21	15-33	11.42

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Table 35. Body lengths of 683 transforming <u>Rana pipiens</u> reared in the laboratory at three temperatures. Larvae with normal spines and crooked spines are compared

T 1 1	<u></u>	N	Normal Spine			Crooked Spine		
Regime	Stage of Development	N	X	SE	N	X	SE	
	41	22	31.9	1.03	5	29.8	1.77	
	42	24	29.2	0.58	7	29.4	1.31	
20 C	43	18	29.2	0.63	11	29.4	0.79	
	44	83	29.9	0.30	20	29.4	0.78	
	45	2	29.0	2.00				
	46	0						
	41	2	23.5	0.50	0			
	42	9	24.6	1.09	7	25.1	0.51	
	43	21	26.0	0.47	5	27.0	0.77	
25 C	44	156	26.8	0.18	19	26.8	0.56	
	45	26	26.0	0.36	3	25.3	0.65	
	46	1	20.0		0			
	41	0			2	28.0	2.00	
	42	2	24.0	1.00	33	24.1	0.58	
	43	6	23.5	0.34	23	23.4	0.43	
30 C	44	42	25.2	0.34	95	24.3	0.24	
	45	9	25.2	0.86	12	22.9	0.60	
	46	0			2	15.5	0.50	

Table 36. Laboratory reared <u>Rana pipiens</u> with normal and crooked spines. Body lengths (mm) of transforming larvae reared at three thermal regimes are compared by stages of development

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DISCUSSION

Field Study: Pitfall Trapping Survey

Pitfall traps provide an effective technique for sampling adult amphibian populations (Gibbons and Bennett, 1974). The two anuran species most commonly encountered in the area: <u>Rana pipiens</u> (the leopard frog) and <u>Bufo terrestris</u> (the Southern toad) account for a majority (61%) of all anurans trapped during the 13 month study period (Table 2). Adding the data for <u>Gastrophryne carolinensis</u> (the narrowmouthed toad) increases the catch of these three dominant species to 86% of all anurans. The remaining 14% of the catch is represented by 10 less prominent species. These data generally conform to the concept that communities are composed of populations of few "common" and numerous "rare" species (Odum, 1971).

Although repeated sampling data for terrestrial anurans probably approximate population values that might be obtained in a census, figures for arboreal species are not as accurate. Adapted to arboreal habits, some representatives of the anuran family Hylidae (especially <u>Acris gryllus</u> and <u>Hyla</u> sp.) possess toe discs that allow them to cling to vertical surfaces. Pitfall trapping data, therefore, underestimate their numbers because they may spend little time on the surface of the ground, and because they can climb out of cans. The numbers of <u>Acris</u> gryllus trapped are especially low. Every month of the year the more

aquatic cricket frogs can be found around the periphery of Pond C Reservoir. They are residents of the grassy shoreline, and their actual numbers should probably be an order of magnitude or more larger. Even if actual densities were larger by several orders of magnitude, however, they would not approach those of the three dominant species.

Pitfall traps effectively measure both localized activity and extended movements. Whereas the frequencies of recaptured specimens are low (usually 3% to 17%), numbers trapped are attributed primarily to movements. Localized activity of few specimens would be characterized by high recapture frequencies. As specimens were not individually marked, some were, doubtlessly, recaptured more than one time. The numbers of recaptures, therefore, are over-estimated for the three most common species. Excluding members of the family Hylidae, low recapture rates among anurans are interpreted as a manifestation of reduced population size in the habitats studied (<u>R. clamitans, B. quercicus</u>) or extreme seasonality (migratory, breeding movements instead of activity: <u>G. carolinensis, R. areolata</u>, Table 2).

The only urodele (salamander) trapped in appreciable quantities (50% of the total 291 specimens) was <u>Plethodon glutinosus</u> (the slimy salamander). The totally terrestrial breeding habits of the species excludes it from interest in the present study.

Rana pipiens--Seasonality

Monthly trapping of <u>R</u>. <u>pipiens</u> demonstrates that the greatest numbers occurred in the spring and early summer (Table 3). The body length at transformation is 20 to 33 mm (Wright and Wright, 1949).

Although frogs the size of mature adults were caught, the mean body lengths for <u>R</u>. <u>pipiens</u> trapped from March to June closely approximated the size of emergent transformees. A listing of the ranges in body lengths show that recently metamorphosed young (<31 mm) were present every month of the year. In February 1972, however, all 11 specimens trapped were recaptures (Table 3).

When total captures are separated into initial captures and recaptures, the proportion of specimens trapped for the first time (initial captures) corresponded to the total numbers trapped, and the mean body lengths of initial captures was consistently smaller than values of total captures. The high proportion of R. pipiens trapped initially each month (low numbers of recaptures) indicates that pitfall data represent directional movement instead of local activity. An average of 17% of trapped specimens were recaptures. Since data do not account for multiple recaptures, recapture estimates are probably somewhat high. A very strong negative correlation (-0.68) between mean body length and numbers of R. pipiens trapped biweekly is evidenced by the short body lengths of leopard frogs sampled during the spring period of peak activity. After July, when numbers of trapped specimens declined, the data demonstrate a higher proportion of adult R. pipiens in pitfalls. Emergent young were trapped after September (as evidenced by the minimum lengths of specimens captured), even though mean body lengths are in the size ranges of adult specimens.

The strong negative correlation between numbers trapped biweekly and body length reiterate that transformees dominate populations in the

spring and adults dominate populations in the late summer and winter. This relationship poses interesting speculation concerning population size structure and the carrying capacity of the environment. Appropriate analysis might reveal a homeostatic level of frog biomass per unit area. There may be a calculable carrying capacity associated with a habitat which can function under large populations of small frogs or small populations of large frogs. The mechanisms and feed back systems regulating such a hypothetical, homeostatic level would be an appropriate topic for further study.

A maximum overall recapture of 301 (17%) of the <u>R</u>. <u>pipiens</u> trapped suggests that most specimens were not encountered repeatedly, and that data represent accurate population trends rather than much activity of a few specimens. Monthly occurrence of immature <u>R</u>. <u>pipiens</u> (Table 4) portrays the pattern of emerging young in the vicinity of the reservoir, and reiterates that continual breeding and metamorphosis occur there.

Since the breeding migration of <u>R</u>. <u>pipiens</u> occurs over a much longer period of time than that of <u>B</u>. <u>terrestris</u>, one cannot accurately determine exactly when eggs were deposited. For this reason it is not possible to test for size differences of emergent transformees among thermally distinct areas. Leopard frogs are known to breed throughout the year in the Southern parts of the country (although primarily in the spring). <u>B</u>. <u>terrestris</u>, however, demonstrates a sudden (explosive) breeding migration that usually occurs within a few nights of heavy rainfall (during March on the S. R. P.). This contention is further

substantiated below (cf., "Reservoir Sampling of Larval <u>Bufo</u> terrestris").

Bufo terrestris--Seasonality

In contrast to the activity and emergence of <u>R</u>. <u>pipiens</u> throughout most of the year, <u>B</u>. <u>terrestris</u> were encountered nine months of the year, and emergent transformees trapped only May to July. Monthly trapping data (Table 5) demonstrate a bimodal activity pattern of migrating adults (March and April) and emergent young (May, June and July). The mean body lengths of initial captures from May to July, considerably lower than other months, give evidence of metamorphosis at that time. The previously marked individuals captured in May to July did not include emergent young. <u>B</u>. <u>terrestris</u> is not highly aquatic. It occurs in large numbers on breeding migrations of adults to and from water and on emergent migrations of transformees.

The low number of specimens recaptured (272) represents the same proportion (17%) found for <u>R</u>. <u>pipiens</u>. Although low, this proportion also is slightly over-estimated due to successive recaptures of some specimens. Since recaptures were few, trapping data are interpreted as a representation of large numbers of <u>B</u>. <u>terrestris</u> (movement) rather than multiple recaptures (activity) of a few specimens.

Gastrophryne carolinensis--Seasonality

Uncommon except during the breeding season from May to October (Wright and Wright, 1949), <u>G</u>. <u>carolinensis</u> were trapped in nine of the 13 months of study. Although third in abundance of 13 anuran species,

<u>G. carolinensis</u> (more terrestrial and more restricted in monthly occurrence than <u>B. terrestris</u>) demonstrated only 3% recaptures. The bimodial activity pattern represents overlapping breeding migrations of adults (June to August) and emergence migrations of transformed young (August to October; Table 6). Since emerging transformees (8.5-12.0 mm; Wright and Wright, 1949) were trapped during four months, <u>G. carolinensis</u> were shown to breed and develop in the vicinity of the reservoir. On several occasions eggs were observed in the reservoir proper and in shallow, temporary rain pools. Although the minimum body length of specimens trapped was 10 mm, sufficient transformees were not caught to allow for a comparison of body lengths among areas or with the literature.

Acris gryllus--Seasonality

<u>Acris gryllus</u> (the cricket frog) is commonly found along the shoreline of Pond C Reservoir. As these small, active frogs easily held onto the sides of pitfalls and escaped the traps, pitfall data do not accurately characterize their abundance (Table 7). They were encountered throughout the 13 month period of study, but remained localized within the emergent vegetation along shore. Specimens were not caught in traps located in wooded areas. Data for specimens that escaped pitfalls are not presented.

Emergent transformees were trapped from July to December; <u>A</u>. <u>gryllus</u> emerge at 9 to 15 mm body length from April to October (Wright and Wright, 1949). Trapping of immatures in Pond C Reservoir did not

represent modifications in breeding habits (from July to December). The low occurrence of recaptures (2.9%) reiterates the inefficiency of pitfalls for sampling this species, as well as the absence of pronounced breeding migrations. Most adults probably remain closer to the water than where pitfalls were placed. The seasonality shown coincides with the normal pattern for the species, not greatly affected by the thermally polluted reservoir.

Pseudacris triseriata--Seasonality

<u>Pseudacris triseriata feriarum</u> (the upland chorus frog) commonly breeds from February until May and emerges until mid-June at 8 to 12 mm (Wright and Wright, 1949). It was not caught in great numbers. Unlike <u>Acris gryllus</u> and <u>Hyla</u> sp., <u>P. triseriata</u> could not easily escape pitfalls. Trapping data are therefore considered to be representative of actual population density (Table 8). A unimodal seasonal activity peak of adults (April to May) precedes emergence of young (June).

The seasonal occurrence of <u>B</u>. <u>terrestris</u>, <u>G</u>. <u>carolinensis</u>, <u>A</u>. <u>gryllus</u> and <u>P</u>. <u>triseriata</u> are comparable with patterns demonstrated by populations elsewhere on the Savannah River Plant where the water is not thermally affected (Gibbons, in preparation). Data presented for <u>R</u>. <u>pipiens</u>, however, demonstrate activity every month, and emergence of transformees for 11 months of the year. Breeding of adults and emergence of transformees therefore occur with greater frequency at Pond C Reservoir than at Karen's Pond or Risher Pond on the Savannah River Plant. Further comparisons are presented below.

Rana pipiens--Distribution

Pitfall catch per trap is presented for <u>R</u>. <u>pipiens</u> (Table 9). Traps are listed in order of position along the thermal gradient (cool to hot), and are grouped according to thermal zones (Table 1, Fig. 2). It is clearly evident that more specimens were trapped at the cool end as opposed to the hot end of the thermal gradient. An overall low frequency of recaptured specimens (17%) substantiates that the high numbers trapped represent many individuals rather than successive recaptures of a few. Variability in catch per trap, however, is very high and numbers do not closely conform to the thermal gradient. High variability among traps is due mainly to positioning effects. As trap sites were systematically spaced at regular intervals along the thermal gradient, they represent an array of habitat types: woods, grassland, marshy seepage areas and several ecotones.

Catch per trap area was highest in pitfalls associated with nearby cool seepage ponds (areas A, B, I, J, and Z) or reservoir shallows receiving cool seepage where larval development was known to occur (areas V and K) (Fig. 2). When reactor activity was periodically discontinued, reservoir water levels declined 0.3 to 1.0 m. At various times, developing larval <u>R</u>. <u>pipiens</u> were secured from the exposed shorelines of the reservoir near trap sites V, W, E, and K; doubtless they occurred elsewhere as well, although undetected. Trapping area Z, although associated with a seepage pond, did not demonstrate a particularly high occurrence of <u>R</u>. <u>pipiens</u>. This site was considerably removed from other ponds; terrain there was xeric, without apparent seepage, and at the hot extreme of the thermal gradient.

Pitfall catch, therefore, represents a combination of variable activity at the different sampling areas, emergence of young from cool peripheral seepage ponds, and emergence of young from reservoir shallows. Total catch of <u>R</u>. <u>pipiens</u> was lowest at trapping sites F, G, H, X and M. Trapping areas F, G, H and X were all surrounded by woods; area M was partly wooded and very xeric. Unfavorable habitats (elevated and wooded) at trapping areas F and G are indicated by the absence of adults caught there. Minimum values of mean body lengths for specimens trapped indicate the presence of emergent transformees at all trap areas. Rather than newly emergent young, however, specimens in some areas must represent the migration of young frogs from other reservoir areas. Nowhere within 50 m of trap area M (for instance) is there an aquatic habitat in which larval amphibians might develop.

Data for initial captures exclude those specimens recaptured. Trap sites associated with seepage ponds or reservoir shallows (A, B, I, J, V, and W) catch the greatest numbers of specimens. Recapture rates for specific traps ranged from 6% (U) to 31% (W). Mean body lengths for recaptures were generally larger than for initial captures. These data suggest that more adults were recaptured than immatures. Two-way breeding migrations would expose adults to a greater likelihood of capture.

Bufo terrestris--Distribution

<u>Bufo</u> <u>terrestris</u> (the Southern toad) was caught most frequently (52%) in pitfall trap areas A, B and C (all in thermal zone I, Table 10). Emergent transformees (5 to 11 mm) were removed only from trap areas A

and B. Although the numbers trapped are much higher in thermal zone I, they do not decline steadily in traps progressing toward the hot extreme of the thermal gradient (Table 17).

<u>B. terrestris</u> and <u>G. carolinensis</u> are less aquatic than other anurans for which data are presented. They are normally located around water in great numbers only during breeding migrations (<u>B. terrestris</u> in the spring, <u>G. carolinensis</u> in the summer). During most of the summer and autumn they remain active and may be encountered even in dry, elevated habitats. Greater numbers trapped at the "cooler" extreme of the thermal gradient (zone I) probably results from the natural drainage there. Elevation there is less than anywhere else along the cool arm of the reservoir. In March 1972, <u>B. terrestris</u> larvae were found and widely collected along the reservoir shore (cf. "Reservoir Sampling of Larval <u>Bufo terrestris</u>"). Specimens are believed to have occurred in many areas where they were not trapped in pitfalls.

Initial captures of specimens were also most numerous in zone I. Very few emergent transformees were encountered; their influence was insufficient to affect the mean body size of specimens trapped. Emergent young were trapped only as initial captures; none were recaptured. The infrequency of captures and absence of recaptures show that newly transformed <u>B. terrestris</u> migrate away from the water and into surrounding terrestrial habitats. Pitfall captures of emergent toads must, therefore, greatly underestimate their numbers. Since they are small and move only short distances at a time, they may well have avoided pitfalls or have been eaten by adults therein. Recaptures (as total captures) were least in trap sites U, V, X and Z. There is no apparent reason for lowest trapping success to occur among traps on the same side of the reservoir. Habitats at these trap sites are not greatly similar. Trapping area V (that caught the highest proportion of <u>R</u>. <u>pipiens</u>) was a site of considerable larval amphibian development (cf. "Reservoir Sampling with Dipnets and Minnow Traps"). Cooled by shoreline seepage there, the reservoir shallows should have provided an especially favorable habitat for larval toads. Trapping area Z was closely related to a nearby seepage pond, but this site also yielded low levels of <u>B</u>. <u>terrestris</u>. If the emergent young emigrated along depressed terrain, however, they would have missed the pitfalls at area Z (which were elevated on a sandy mound).

A high water table and considerable seepage at site V necessitated placement of pitfalls at greater distances from the reservoir than elsewhere. As the development of <u>B</u>. <u>terrestris</u> there is documented below, the low occurrence of specimens in trapping area V is attributed to positioning of pitfalls outside natural emigration routes. Dense brush was located directly in front of the pitfall-drift fence section at area V. Emigrating young probably follow the shoreline until a suitable pathway is found.

Although the body lengths of newly transformed <u>R</u>. <u>pipiens</u> did not differ from the values reported by Wright and Wright (1949), <u>B</u>. <u>terrestris</u> did not conform to their data. The authors list the body lengths of recently metamorphosed <u>B</u>. <u>terrestris</u> as 6.5 to 11 mm. Many specimens trapped in pitfalls were as small as 5 mm (Table 10). Data

are not sufficient to test the statistical significance of this apparent difference. The minimum body lengths of <u>B</u>. <u>terrestris</u> removed from Karen's Pond (Table 21) was 7 mm, which is closer to the expected size range. Although the smaller size of <u>B</u>. <u>terrestris</u> from Pond C Reservoir might be explained by random variation, data suggest that the body size of newly transformed toads in the vicinity of the heated reservoir is less than might be expected.

Gastrophryne carolinensis--Distribution

<u>Gastrophryne carolinensis</u> (the Eastern narrow-mouthed toad) was also trapped in all pitfalls. A majority (52%) of the specimens occurred in pitfall areas A, B, C and Z. They were not equally dense in all traps near seepage ponds, but 25% occurred in trap area Z (near a cool seepage pond there, Table 11). Emergent young were trapped in pitfall areas A, U, C, V, H, J and Z. Habitat types at these sites are highly variant. The high proportion of transformees caught at area Z is expressed in the very low mean length there.

The low overall recapture rate (2%), as well as that for trapping area Z (3%), demonstrates the explosive nature of breeding migrations in <u>G</u>. <u>carolinensis</u>. Of the anuran species discussed, <u>G</u>. <u>carolinensis</u> manifests the shortest seasonal activity (nine months), remaining around the water for the shortest period of time (Table 2). Because of the high occurrence of specimens at trap area Z, any direct conformity between the thermal gradient and numbers trapped is obscured.

Dominant Anurans--Distribution

The numbers of specimens caught per trap area among the three dominant species of anurans exhibit a general trend of more specimens at the "cool" end and fewer specimens at the "hot" end of the thermal gradient (Table 12). The highly aquatic <u>R</u>. <u>pipiens</u> shows this trend best, followed by the more terrestrial <u>B</u>. <u>terrestris</u>. Total catch is greatest in zone I for all three species. Data for <u>G</u>. <u>carolinensis</u>, however, disrupt the gradient response shown by the other two anurans. The combined numbers of the three dominant species do not show a clear gradient response, although the greatest numbers (in overall ranking) were caught in the trap areas at zone I (A, B, C) and the fewest in a trap area in zone VI (L).

Localized effects of terrain and habitat greatly influence anuran responses to the thermal gradient. Overall values for zone III are especially low because of wooded trap areas F and G. Ranking of traps by capture success for the three anuran species demonstrates these differences, and result in a disordered array of overall ranks. These differences are due to location effects: variable affinities for shoreline areas V and W, semi-wooded area U, and cool seepage pond Z particularly.

Analysis of the thermal gradient, therefore, is shown to be difficult even though the temperature differential is very real. Localized effects of specific trap habitats and chance occurrence of a species in any given seepage pond considerably influence overall numbers per trap.
Acris gryllus--Distribution

As already mentioned, pitfalls are less effective for sampling most tree frogs and their relatives (family Hylidae) than other anurans, because they possess expanded toe discs that are adapted for clinging to vertical surfaces. The Southern cricket frog (<u>Acris gryllus</u>) was the only major anuran subject to this trapping bias. Although <u>P. triseriata</u> is also a member of the same family, it possesses reduced toe discs that do not allow specimens to escape by scaling the vertical surfaces of pitfalls. <u>A. gryllus</u> would often leave the pitfalls before they could be measured and recorded. Although the actual numbers trapped are known to be greatly underestimated, the relative abundance among traps should represent valid comparisons. Specimens should have escaped from all pitfall traps with equal ease.

Traps catching the highest numbers of specimens (I, J, W, E) are similar in placement near littoral, emergent vegetation (Table 13). Although traps I and J are both associated with cool seepage ponds, trap Z (also near a seepage pond) was approximately 2 m removed from aquatic vegetation, and caught few numbers.

<u>A. gryllus</u> remains localized in vegetation along the reservoir or seepage ponds throughout the year. Traps away from vegetation (as at wooded sites F, G, H, X, Y) caught few specimens. Positioning of the pitfall at site V from the shallow shoreline resulted in few specimens being trapped there also. These data, along with a low recapture rate (3%), reiterate the reduced mobility exhibited by this species.

Emergent young (9-15 mm) were encountered throughout the gradient. Because of the extensive shoreline seepage along the reservoir and the limited mobility demonstrated by <u>A</u>. <u>gryllus</u> within these areas, the species is not considered to be as severely affected by the thermal loading as wider distributed anurans having larger, more mobile larvae. Adults continuously litter the shoreline along the periphery of the cool arm of Pond C Reservoir. The monthly sampling program revealed that larvae of <u>A</u>. <u>gryllus</u> did occur in parts of the reservoir proper (cf. "Reservoir Sampling with Dipnets and Minnow Traps").

Pseudacris triseriata--Distribution

Pitfall catch for <u>P</u>. <u>triseriata</u>, the upland chorus frog, was least among the five dominant anuran species trapped (Table 2). The low numbers of specimens trapped (172) and the low recapture frequency (4%) suggest that the distribution of this species is less extensive than other anurans. Larger numbers of specimens trapped at area Z may well indicate a preference for cool ponds over the heated reservoir. Lower numbers of chorus frogs at other pitfall trapping areas (A, B, I and J), where cool seepage ponds also occur, however, indicate that reproduction is extremely localized (Table 14).

Since emergent transformees were trapped at area Z, they are known to have completed development there. Emergent young were also caught at pitfall trapping area A (where few specimens were encountered). Larger numbers of specimens caught at pitfall site V and the low overall

mean body length of specimens trapped there suggest that <u>P</u>. <u>triseriata</u> also may well breed and develop in Pond C Reservoir.

Immature Rana pipiens

To analyze the occurrence of immature R. pipiens along the thermal gradient by season, the numbers of immatures (< 47 mm body length) initially caught per trap are listed by month (Table 15). The symbol (*) indicates the presence of at least one recently transformed specimen (< 30 mm). The high negative correlation already shown between the numbers of specimens trapped biweekly and their body lengths is sufficient evidence to assume that the presence of many immature larvae represents large numbers of emergent transformees. This is shown to be true. Only four monthly records of more than 10 captures per trap area fail to include transformees: trap A for July, trap C for February and June, and trap E for April. The July catch at trap site A contains no specimens < 30 mm, although they were encountered there during the two months before and month following. This does not mean, however, that the specimens trapped did not include transformees. As already stated, Wright and Wright (1949) report the body lengths of newly metamorphosed R. pipiens as 30-33 mm. Many of the 57 immature specimens trapped were within this range. To assure that specimens were indeed transformees, a conservative criterion (< 30 mm) was used to identify transformees. The monthly totals of immature R. pipiens caught per trap certainly included larger transformees. None of the figures indicated by the symbol (*) (Table 15), however, can be doubted to represent recently metamorphosed frogs.

The catch for February and June at site C and for April at site E do not include specimens <30 mm, although transformees were trapped in adjoining months. Either the metamorphosed larvae were actually not encountered or they were larger than those elsewhere. Larvae laboratory-reared at cooler temperatures have been shown to attain larger body size than those at warmer ones (Etkin, 1968; cf. "Rearing of Larval Rana pipiens").

Although overall peak metamorphic activity was demonstrated from May to July, larvae have been shown to occur year around at certain trap sites. Emergence of young at trap area Y continued for seven successive months. The pitfalls at site Y are very close to the reservoir proper. Considerable seepage into the reservoir allows development of young there. The absence of other breeding ponds at trap area Y indicates that frogs did emerge from the reservoir proper.

Although trap area Y is located near the hot extreme of the thermal gradient (Fig. 2, Table 1), the shoreline there is cooled by a localized pothole of underwater seepage. When the reservoir water level drops >6 cm, it exposes a protective shelf of sand that gives the appearance of an atoll (Fig. 12). The success of larval development there is extremely probabilistic, depending upon a combination of stresses: fluctuating water level and critical thermal tolerance (reactor activity). Because of increased developmental rates at elevated temperatures, emergence at Y early in the year may represent more rapid development there, or earlier breeding there. When compared

to the heated reservoir, cool seepage ponds might well retard developmental rates but cause larvae to grow and metamorphose at a larger size. The absence of emergent transformees at trap area A in July might well represent the increased size of larvae reared at cool temperatures.

Body lengths of newly transformed <u>R</u>. <u>pipiens</u> trapped in pitfalls were apparently not different from the size range reported for transformees by Wright and Wright, 1949 (22-33 mm). Although the body size of recently metamorphosed frogs may be influenced by the increased temperatures of the heated reservoir, data from the pitfall study do not demonstrate such an effect. Minimum body lengths recorded for <u>R</u>. <u>pipiens</u> are not smaller than the values reported by Wright and Wright (1949).

Cool seepage areas (A, B, I, J, Z) demonstrate the occurrence of numerous transformed larvae. Traps surrounded by dry woody habitats (D, F, G, H, M) caught fewer young overall and much fewer emergent transformees.

The Gradient Effect

Since the temperatures in the cool arm of Pond C Reservoir may vary from lethal (near 50 C) at the mouth to ambient at the opposite extremity, one might expect the distribution of specimens in the designated thermal zones to reflect the degree of stress sustained along the shoreline. It was of interest to determine whether or not adult or immature anurans occur uniformly along the heated reservoir.

To summarize population responses to the overall thermal gradient, the mean numbers of the five dominant species trapped in pitfalls were compared among thermal zones (Tables 16-19). Data represent the mean numbers of anurans trapped among pitfalls at each zone. Probabilistic breeding success among species at certain peripheral seepage ponds and variant habitat requirements have been shown to be sufficient to distort generalized clinal phenomena. Local conditions at specific trap sites along the thermal gradient appear to elicit responses from the anurans studied that override to some degree, the manifestations of the thermal gradient.

Although numerous recently transformed <u>R</u>. <u>pipiens</u> were caught at protected area Y in February and March, 1971 (Table 15), none were trapped during the same months of 1972. The effect of conditions there (degree of seepage and protection conferred by a surrounding sandy shelf) are doubtless influenced by the reservoir water level (Fig. 12). Successful development of larvae there occurs only because of conditions that protect the microenvironment from otherwise lethal temperatures. Doubtless the possibility of increased reactor activity (resulting in increased temperatures) makes the probability of successful development there low. The occurrence of favorable conditions such as these (though tenuous) disrupt the continuity of an otherwise well delineated thermal gradient. Unpredictable occurrences of this sort are wherein lie the hope of organisms to otherwise uninhabitable ecosystems.

Adults of all five dominant anuran species were trapped throughout the periphery of Pond C Reservoir. The occurrence of R. pipiens,

<u>B. terrestris</u> and <u>A. gryllus</u> was lowest at the hot extreme of the thermal gradient (zone VI). In the same zone, however, especially high numbers of adult <u>G. carolinensis</u> and <u>P. triseriata</u> were trapped (as were most of the few immatures of these species). These data probably represent random, successful breeding there. Receiving little visible shoreline seepage, cool pond Z became virtually dry when the water level of the reservoir dropped appreciably. Neither was the pond ever contiguous with the reservoir. Probably for these reasons, cool pond Z was unique in having no fish. Larval anurans therefore might be free of predatory pressures that occur in all other aquatic habitats in the vicinity. Due to supposed bad taste, larval anurans are believed not to be subject to predation. Palpitated specimens of the snake genera <u>Natrix</u> sp. and <u>Thamnophis</u> sp., however, reveal that larval anurans naturally comprise part of their diets. Bluegill and immature largemouth bass might well feed upon larval anurans.

Because of unexpected successful development of <u>R</u>. <u>pipiens</u> at protected area Y (zone V, Fig. 2), leopard frogs were most numerous in pitfalls at zones I, II and V. Initial captures of immature <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> demonstrate an occurrence frequency virtually identical to that of the adults. As might be expected, the young emerge in greatest numbers at zones where most of the corresponding adults were found. If many adults had been trapped in migration at the reservoir, yet no young were encountered, then data might circumstantially suggest embryonic/larval mortality. Data do not support such a contention; perhaps they are insufficient. Severe embryonic mortality was documented at reservoir area K: <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> (cf. "Reservoir Sampling with Dipnets and Minnow Traps" and "Reservoir Sampling of Larval <u>Bufo</u> <u>terrestris</u>").

As already indicated, the greatest numbers of <u>G</u>. <u>carolinensis</u> were caught at trap area Z (zone VI) which was adjacent to prairie-like terrain. Numerous young emerged from nearby cool pond Z. The preponderance of specimens around the hottest thermal zone, therefore, is interpreted as an isolated opportunistic occurrence rather than a response to the reservoir's thermal conditions there (which were usually supralethal to animals). Pitfall trapping data for <u>P</u>. <u>triseriata</u> and <u>A</u>. <u>gryllus</u> are insufficient to compare trends among adults and initially captured immatures, but as with other anurans, adults were trapped through the study area.

Body Lengths of Pitfall-Trapped Bufo terrestris and Rana pipiens on the Savannah River Plant

Body lengths of specimens trapped in pitfalls are compared among three habitats on the Savannah River Plant for the two dominant species (Tables 20 and 21). Although the months of greatest activity were similar for both species, emergent young were consistently trapped earliest at the heated reservoir. In addition, the body lengths of emergent young were consistently smallest at the heated reservoir for both species. Such a consistent trend between the two anurans alludes to possible growth inhibition in the heated study areas. To be conclusive, data must relate body size to age; there is no technique, however, to age amphibians. The size differences observed might represent random variation. Nonetheless, the minimum lengths recorded are assumed to represent the size of transformees.

Data summaries represent striking similarities among the three habitats. Other than the presence of smallest transformees at heated Pond C Reservoir, the mean body lengths and variances are strikingly similar for both species. The absence of recently metamorphosed <u>B</u>. <u>terrestris</u> at Risher Pond is attributed to predation by fish and low densities there. Considerable variation may occur among years, but data do not show whether apparent size differentials are real or artifact. Unfortunately, data are not available from all areas for the same year.

Field Study: Reservoir Sampling with Dipnets and Minnow Traps

Larvae of nine amphibian species were identified among the 15 consecutive monthly dipnet samples and in minnow trap samples taken in and around the heated reservoir. Cumulative samples included at least one specimen of four anuran larvae (<u>R. pipiens</u>, <u>B. terrestris</u>, <u>A. gryllus</u> and <u>H. cinerea</u>) in all areas. Species diversity was greatest in pond Z, which was never contiguous with the reservoir proper, and least in heated reservoir area K. Very few specimens of the two salamander species were ever encountered.

Because of certain differences among sampling areas, they are not amenable to rigorous quantitative comparisons. Most data presented, therefore, are qualitative. The peripheral cool ponds (Z, A, I and B) were well circumscribed bodies of water that differed in size less than

an order of magnitude. The shoreline at reservoir area V, however, was considerably larger. Although maximum water depths at the ponds were generally not greater than 0.5 m, they were by no means uniform. Different water volumes, amounts of seepage, and vegetation types also distinguish the areas from one another and precluded a strictly quantitative comparison. To prevent the removal of entire populations where species diversity was low, only a few representative specimens were removed. Generally, specimens could be identified in the field, so that not all larvae needed to be preserved.

An expression of quantitative comparisons is evident in the mean numbers of species removed per monthly sample. Cool pond Z revealed an average of 3.1 species per month, whereas faunally depauperate, hot reservoir area K revealed 0.6 species per month: less than one species per monthly sample. Other areas sampled revealed densities intermediate to these two extremes. Another quantitative expression is found in the number of samples in which no larvae occurred. Data show that amphibian larvae of some species (usually R. pipiens) were always present in cool ponds Z, A and I. Over half (57%) of the samples taken at heated area K, however, revealed no specimens. These data attest to the thermally stressed conditions there. Although eggs of G. carolinensis were sampled at area K, no larvae were ever observed. Presumably all specimens in area K died. Although species diversity over the 15 month study period was identical for sampling areas B and V, much greater numbers were encountered at cool pond B than at reservoir shore V. These differences are not apparent from the summarized monthly data (Table 22).

Comparing the monthly samples of larval amphibians among the six areas, species diversity at non-contiguous cool pond Z is 100% greater than corresponding figures from heated area K and 25% greater than those from heated area V. Data for the other cool ponds (occasionally contiguous with the reservoir) fall somewhere between these two extremes. Over the 15 month study, the average number of species trapped per month at pond Z was 500% greater than heated area K and almost 300% greater than heated area V, with intermediate values for the other cool areas. In addition, more than half of the monthly samples taken at heated area K and depauperate cool pond B revealed no specimens. All other areas yielded at least one species for each monthly sample.

The disparity of data between areas Z and K are outstanding. Greatest overall results (diversity and commonness) at area Z may well be partly due to the absence of fish there. The absence of elevated temperatures from reservoir overflow at pond Z doubtless contributes to stability and resulting greater diversity. Sparse samples at area K are interpreted as a result of thermally stressed conditions there. The minnow traps continuously maintained in all areas during the same 15 months, provide comparable data. All anuran species caught in minnow traps were also removed in monthly dipnet samples.

<u>Lepomis macrochirus</u> (bluegill) were minnow trapped in all areas except pond Z. Specimens captured at heated area K usually died in the traps. Fish and anuran larvae demonstrate an inability to withstand

sustained high temperature levels. Massive fish kills as noted at area K did not occur elsewhere. Embryonic and larval mortality for <u>B. terrestris</u> and <u>R. pipiens</u> were observed several times. Except in shallow cool seepage areas, temperatures at area K were apparently lethal except during periods of heavy rainfall.

Sampling data reveal greatest species diversity, population sizes, and frequency of occurrence (commonness) of larval amphibians at cool pond Z. Other cool ponds were less favorable, and heated reservoir areas considerably less favorable to larval amphibians. The vast differences observed among areas are partly indicative of thermally stressed conditions in the heated reservoir.

Total Lengths of Larval Rana pipiens

Many specimens removed from minnow traps and taken in the 15 successive monthly samples were measured (total length, mm) and staged according to the level of development (Gosner, 1960). To determine whether size differences were related to the thermal regime from which larvae were removed, larval lengths are compared (by specific developmental stage) for each of three sampling areas (Fig. 13). Pond Z was cool, unaffected by thermal effluent. Shore areas V were characterized by higher temperatures (cf. Table 1, Fig. 2). The fewest numbers of specimens were removed from the hottest area (K), where larvae were not commonly encountered. Most larvae trapped there were removed, yet data for only 27 larvae were available. Since specimens were always available at cool pond Z, few were removed. At intermediately heated area V, however, most specimens were removed. Low survivorship of larvae at heated area K reduced the probability of capturing specimens a second time. Therefore, they were not released.

At all stages of development, larvae removed from the hottest area K were smallest. Larvae removed from cool pond Z were usually the largest. Small sample sizes may account for inconsistencies at advanced stages 35 to 38. Overall larval lengths are reduced in these stages as the larvae near transformation (stage 46). Differences, therefore, may be less distinct.

Sample sizes are small at some stages for sampling area K, and inconsistencies should be expected. The overall trend, however, shows largest larvae in the coolest area (B) and smallest larvae in the warmest area (K). These data support the contention that growth in amphibian larvae is inversely related to temperature (within the range of temperatures studied).

Field Study: Reservoir Sampling of Larval Bufo terrestris

Larvae sampled in this study are believed to have originated primarily from the massive spring breeding migration prior to 22 March 1972. Concomitant with the greatest monthly rainfall (2.3 cm), a record number of adult <u>B</u>. <u>terrestris</u> (562) were trapped in pitfall traps around the reservoir on this day. Data from another pitfall study of amphibian activity conducted the same year at Karen's Pond (a 0.1 ha vernal pond on the Savannah River Plant, 13.0 km from the reservoir), revealed the greatest yearly catch of <u>B</u>. <u>terrestris</u> on the same day. Pitfall captures (Table 23) indicated that little movement occurred on other days. Successive samples, therefore, were from discrete populations at each location except K (Table 24), and comparisons could be made of stages and lengths as functions of time and temperature.

As already mentioned, high egg and embryonic mortality is the overriding effect of thermal loading in hotter sections of the reservoir. Area K was the most severely stressed location from which larval amphibians were removed in any of the sampling programs. Initial (>95%) heat death of <u>B</u>. <u>terrestris</u> eggs among three clutches there on 29 March and 17 clutches of <u>R</u>. <u>pipiens</u> on 22 January document the great extent of embryonic mortality. Since only living embryos were encountered elsewhere along the reservoir, severe mortality at area K is attributed to heat death. All viable embryos at area K occurred in egg masses situated where cool stream seepage entered the reservoir, cooling temperatures there. Ruibal (1959) reported similar marginal survival of Rana pipiens eggs in seepage areas of hypersaline desert streams.

The presence of dead embryos at heated site K suggests either that adult toads deposited eggs in water temperatures exceeding their maximum thermal tolerance (33 C; Volpe, 1953), or that they deposited eggs in water only temporarily at sublethal temperatures. Hathaway (1928) reported that larvae of <u>Bufo americanus</u> could withstand a maximum temperature of 36.3 C for 24 hours. Heated effluent entered the reservoir continuously for several days before the appearance of eggs at area K. Although the eggs were deposited within a temperature range

of 29-36 C, the temperatures probably remained closer to 36 C, falling to 29 C only during heavy rainfall.

On the night of the massive breeding migration, the cooling influence of rainfall did permit initial survival of larvae. Many, however, died when lethal thermal conditions were restored. Mean March water temperature fluctuations (maximum-minimum) recorded at area K during rains were 14 C, as opposed to 8 C when no rainfall was recorded. Samples from 29 March and 5 April attest to the survival of a small number of embryos from clutches laid subsequent to 22 March (Table 24). The reduction from 29 March to 5 April in both stages and lengths of the two samples from site K indicates that they represent two distinct cohorts each week. The absence of successive samples from a single population at area K is interpreted as a manifestation of thermal stress and attests to larval mortality, in addition to that observed in embryos. Since only living larvae were encountered elsewhere in the reservoir, extreme mortality at area K is attributed to heat death.

Within the range of thermal tolerance at the other areas, both lengths and stages of larvae sampled reflect the level of thermal loading sustained. In the first sample (29 March), both lengths (Table 25) and stages of development (Fig. 14) differed significantly among the three areas. Stages from area B were appreciably ahead of the others in development as eggs were deposited there earliest of all. Toads migrating to the reservoir would be expected to breed in peripheral ponds before reaching the reservoir proper. More rapid development in warmer sites A and V allowed individuals in successive samples from

these areas to surpass larvae at site B in size and stage. Mean stages and lengths did not differ significantly among the three sampling areas in the second weekly sample (5 April) due to this reordering. Larvae from the three reservoir stations on 5 April clearly demonstrate increased development and growth among larvae in heated parts of the thermal gradient. Within two weeks of development at area D, larvae were nearing transformation (stage 46).

Specimens from area B were consistently larger than those from areas A or V at comparable stages of development. The test data strongly suggest that metamorphic lengths attained by larvae reared at cool temperatures are significantly larger than those attained in the areas receiving the influence of heated effluent (Table 26). Fish and larval amphibians maintained at lower temperatures in the laboratory have been shown to develop more slowly and to attain a larger size than those at warmer temperatures (Etkin, 1955; Kokurewicz, 1969; Muto, 1972; Wilbur and Collins, 1973). Variation due to measuring is greater for toad larvae than for larger anuran species (<u>e g. Rana pipiens</u>). If the lengths of metamorphic toads were larger, the size differences among sampling areas would likely be more clearly demonstrated.

The adaptation of anurans to nocturnal breeding migrations during rainfall fortuitously confers a double advantage to survival in a thermally polluted reservoir. Not only are nocturnal temperatures lower than those during the day, but substantial amounts of rainfall that commonly accompany anuran breeding migrations further reduce water temperatures in the reservoir. Toads lay eggs at night or early morning

when water temperatures are low. Embryos thereby pass through the most thermally sensitive stages (uncleaved egg and gastrulation; Atlas, 1935; Grainger, 1959; Hoadley, 1938; King, 1903; Moore, 1942b; Volpe, 1953) before water temperatures increase and approach the maximum temperature tolerance of early embryos (Brown, 1967; Zweifel, 1968). Anurans depositing eggs in a heated reservoir, however, are denied one of the inherent advantages of breeding in reduced nocturnal temperatures. High egg and embryonic mortality, therefore, is the obvious overriding effect of thermal loading in hotter sections of such a reservoir.

Data from these natural field populations corroborate trends demonstrated among larval amphibians in the laboratory. Since conditions prevailing in field situations are much more variable than those maintained in laboratory experiments, thermal responses of organisms and populations in nature are not as easily documented. Since field conditions are not nearly as well controlled as those in the laboratory, statistical significance is not as easily documented. Variability in field conditions at the study areas probably accounts for the fact that only two of three analyses were statistically significant. Although water temperatures were monitored at specific locations, toad larvae are known to thermoregulate behaviorally. They have been shown to selectively aggregate in warmer areas of a pond. Perhaps they also select cooler, optimum temperatures. Brattstrom (1962, 1963) documented several cases of behavioral thermoregulation in larval anurans and discussed its adaptive significance. For this reason, field studies are needed to determine the extent to which thermal parameters derived from

the laboratory can be extrapolated to actual conditions in nature. Field data should be instructive in determining thermal limits that can be imposed on biological systems without destroying stability.

In addition to the overriding effect of outright heat death, precocious development and retarded growth in thermally loaded areas, receding water levels, predation and shortages of food and cover could impose other threats of mortality. During the period of time that the toads were developing in the reservoir, cessation of flow from the thermal canal probably would have resulted in the death of all larvae except those in cool pond B or the inland pool at site V. Reactor shutdown results in receding water levels that leave larvae exposed on dry ground. The biological effects of fluctuating water levels are discussed by Kroger, 1973.

Since temperatures in the reservoir exceed the thermal tolerance of most organisms, blue-green algae comprise most of the flora, with the exception of scattered patches of green algae (<u>Spirogyra</u> sp.). The sterile vegetative conditions in the reservoir proper provide little food and cover for anuran larvae. Starvation and predation, therefore, are likely secondary sources of mortality. Predaceous birds (kingfishers, herons, egrets) are residents of the reservoir and have been observed feeding along the shore where larval <u>B</u>. <u>terrestris</u> occurred. Banded water snakes (<u>Natrix sipedon</u>) and eastern garter snakes (<u>Thamnophis sirtalis</u>) were also trapped along the reservoir. Both are known to prey upon larval amphibians.

Laboratory Study: Rearing of Larval Rana pipiens

A full report of specimens (Table 27) shows the fate of the 1,600 original embryos. Data for each replicate tank were categorized into four classes: accidental deaths, "natural" mortality, transformation through late metamorphic stages, and specimens unaccounted for. Each group is discussed separately below.

Accidental Deaths

The major cause of accidental, experimental mortality was inadvertent mashing of larvae with either the stainless steel siphon covers or weights that stabilized undergravel filters. Increased precautions reduced subsequent accidental deaths from initially higher levels. Large rocks used to hold down undergravel filters (Fig. 11) were replaced with culture dishes (Fig. 10). Since the larvae could be viewed through the glass culture dishes, specimens weren't crushed beneath these weights as they were with the rocks. For unexplained reasons, certain aquaria were slightly more hazardous to larval survival than others (e.g. 20 C--Tank C, 25 C--tank B; Table 27). Accidental deaths were lowest (0.5%) at 35 C where the short-lived embryos were subjected to very little laboratory manipulation in the nine days that some survived. In the three other thermal regimes, accidental deaths were similarly low (ranging from 4.0% to 5.2%). Variability among the three warmer temperatures is attributed to randomness; the total loss to accidental deaths (N = 58, 3.6%) is considered negligible.

Mortality

Observed mortality was greatest (313, 78.2%) in the group of embryos reared at 35 C. Since no specimens survived for more than nine days, mortality is known to be complete, although not fully accounted for. Only two specimens were accidentally killed within this short period of time; others were lost to causes discussed below. Volpe (1953) reports the maximum thermal tolerance of Rana pipiens as 34 C. Although some embryos can sustain limited exposure to 37 C (Briggs, 1947), high mortality at 35 C for an extended period is not surprising. Temperatures approaching the maximum thermal tolerance of a species may be lethal if prolonged through time. Embryonic amphibians are known to be thermally sensitive in certain embryonic stages (early cleavage and gastrulation), but more thermally tolerant in advanced stages. Neither of the "egg" clutches in replicate tanks A and B (with larvae introduced at stage 11: "mid-gastrula") or tanks B and C (with larvae introduced at stage 18: "muscular response") proved to be appreciably more thermally tolerant.

Mortality was second greatest (134) at 30 C. Somewhat reduced mortalities at 20 C and 25 C (112 and 111, respectively) might suggest that thermal stress was greater at 30 C than either of the two other temperatures. Although this might be true, the differences among the 12 replicate aquaria are not statistically significant ($X^2 = 17.96$, P > 0.05).

Transformees

A majority (57%) of the 1,200 specimens introduced into aquaria at 20 C, 25 C and 30 C did develop into late metamorphic stages. The numbers of larvae transforming in the 12 replicate aquaria at these three thermal regimes were not significantly different from one another $(X^2 = 18.63, P > 0.05)$. The differences in numbers observed, therefore, can be attributed to random variation. Early in the study, specimens were removed, measured and preserved upon reaching developmental stage 45 or 46. Mortality at these stages was high initially, and many transformees at stage 45 or 46 were found dead in the tanks. Since they were believed to have drowned, subsequent transformees were removed at an earlier stage of development (mostly 44).

Fifty-two specimens were removed at stage 45 and only three specimens were removed at stage 46. Few specimens, therefore, remained in the tanks through final metamorphosis at stage 46, and the occurrence of dead transformees was virtually eliminated. Larvae of stages 43 to 44 were frequently found to have emerged from the water, holding onto the screen siphon covers. Occasionally, <u>Rana pipiens</u> in the field were caught in pitfalls at stages prior to stage 46 (having naturally emerged from the water).

Larval mortality among anurans has been reported to be greatest at uncleaved egg and gastrulation (Atlas, 1935; Grainger, 1959; King, 1903; and Volpe, 1953) and metamorphosis (Dent, 1968). The results of these experiments corroborate high mortality at transformation. This occurrence, however, may well represent a response to laboratory rearing

and may not be observed in nature. Assuming that mortality at late metamorphic stages is an experimental aberration (not representing natural mortality), specimens dead at stage 45 or 46 were considered as transformees and not attributed to mortality. Had the larvae developed in the field, presumably they would have already left the water by the time they reached stage 45. Growth in <u>R</u>. <u>pipiens</u> ceases at stage 42; final body size is attained by this time (Etkin, 1964), although the tail is continuously resorbed until metamorphosis is complete at stage 46. Larval body lengths, therefore, are considered to be comparable at all stages thereafter.

Missing Data

Data for the four replicate aquaria (A, B, C, D) were similar among temperatures in all classes (accidental deaths, mortality and transformations) except that for which no account of specimens could be given (Table 27). Numbers of larvae in this category were greatest at 35 C and 20 C, and considerable variability was evident at these temperatures. The numbers of larvae were significantly different among the 12 aquaria ($X^2 = 72.93$, p < 0.005) in which larvae survived more than nine days. Of the 400 embryos originally placed in aquaria at 35 C, 21% were not accounted for. Early embryonic stages are small and specimens may have been immobilized in the screen wire of siphon or heater covers. Decomposition occurred much more rapidly at 35 C than at other thermal regimes, and checking specimens twice a day may not have been sufficient to provide an accurate record. Dead or dying larvae were at times found entangled in the spinach, and could have been removed accidentally.

Excluding the group maintained at 35 C, the number of unaccountable specimens appears to be negatively related to temperature (or more appropriately, negatively related to development time, which is shown to be a function of temperature; Fig. 15). Larvae maintained in the laboratory for longer periods of time would have been exposed to a greater chance of loss than those transforming more rapidly. Specimens were lost due to several factors. A few active specimens were able to get inside the stainless steel, siphon screen covers. Not protected there from the current pull of interconnecting siphons among replicate tanks (Fig. 10) or incurrent siphons for the external filters, some larvae were doubtless drawn in, immobilized, and died in the siphons, tubes or external filters. Extended development time of larvae at 20 C probably increased losses there by exposing larvae to repeated, increased amounts of physical manipulation. Possible debilitating effects of inhibited development at 20 C will be discussed below. They probably represent manifestations of stress that result from decreased temperatures.

Larvae demonstrate a preference for close quarters (in corners, below spinach, behind siphon screens, <u>etc</u>.) and occasionally managed to maneuver themselves below the undergravel filters. Specimens recovered dead were not numerous, and were considered "accidental deaths"; these data were deleted from analyses. Rarely larvae were accidentally removed from tanks with old spinach. Usually they were returned to aquaria unharmed; at least one active larva, however, went down the drainpipe of the sink.

Doubtless some specimens for which data were not available were lost to these and other hazards. Two additional transformed specimens were found on the floor of the laboratory on 23 November 1971. Apparently they had escaped the unidentified tank(s) through the corner openings in the plexiglas aguarium cover (Fig. 10, Table 27). They probably escaped from the series maintained at 30 C or 25 C, since these two groups were transforming at the time. Other larvae may have escaped unnoticed as well. Once escaped specimens were discovered, the cover openings were occluded with paper toweling throughout the remainder of the experiment. The total number of escaped transformees is believed. therefore, to have been small. Unaccountable specimens were considerably reduced (104, 9% of 1,200) in the three thermal regimes in which larvae did transform. The majority (66) of these, however, were missing from the group reared at 20 C. The prolonged, 17-month development time for some larvae at 20 C is believed to be responsible. Larvae in this series were maintained for more than twice the development time at either of the two warmer temperature regimes. The time required for R. pipiens to complete normal development reportedly requires 90-120 days (Etkin, 1968), 75-90 days (Rugh, 1951), and 67-86 days (Wright and Wright, 1949). Repeated activity of daily laboratory maintenance (feeding larvae, removing spinach, filtering water) subjected the larvae at 20 C to the greatest probability of loss or accident. Doubtless some larvae were inadvertently removed or injured in the mechanical process of moving weights, siphon screens, etc. For unknown reasons, some replicate tanks in a series (20 C--tank D, 25 C--tank A) had very low incidences of unaccounted-for specimens (Table 27).

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Accidental mortality and unaccountability of data were considered to be unbiased effects, essentially random in nature. Excluding these figures in a comparison of transformation and mortality, therefore, 30-37% of the larvae were lost to mortality and 63-70% transformed (Table 28). Specimens unaccounted for and accidental mortalities are deleted from further comparisons among groups or temperatures.

Growth and Transformation

Mean body lengths and numbers of larval R. pipiens transforming monthly (Fig. 15) are shown to change through time. Although all specimens were placed in aquaria on 2 September 1971, this month is not represented on the figure. Emergent young (transformed larvae) were not secured until October. As indicated on the figure, the final samples in series 20 C and 30 C were both terminated before all larvae transformed. A malfunctioning thermoregulator allowed water temperatures in the 30 C group to exceed 40 C on 11 February 1972; the eight larvae remaining were thus killed. Presumably the larvae would have completed development through final metamorphosis within the following month (March). The series maintained at 20 C was deliberately terminated after being maintained for 18 continuous months without transforming. The fact that 21 specimens still survived at the 20 C series after 18 months suggests that larvae there might have persisted for several weeks or months more. Although these terminated larvae were not completely transformed, most were developed sufficiently to indicate their body lengths at metamorphosis. Larvae reared at 25 C transformed or died without interruption. Lengths of transforming larvae reared at the three temperatures are compared below.

Two major points are immediately evident. Pronounced size disparities in body length were exhibited among larvae reared at the three different thermal regimes. Secondly, outstanding differences lie in the extended amount of time required to complete development at the coolest temperature regime (20 C). Although the larvae maintained at 25 C required less than two additional months to develop than those at 30 C, 21 of those larvae reared at 20 C were arrested after >10 additional months and may have required considerably more time. Development at 20 C therefore was quite depressed in comparison. (Survivorship is estimated above.) Conditions responsible for this protracted development may be in part due to two factors: first the unusually low temperature and secondly in the constancy of the temperature cycle. Larval development at constant temperatures has been shown to be slower than at fluctuating regimes. Rana pipiens may develop more rapidly when exposed to temperature fluctuations than at constant temperatures, even when the temperature of the constant thermal regime equals the maximum temperature achieved by the fluctuating cycle (Ryan, 1941).

Similar to other biological and chemical processes, development is known to vary with temperature. In addition to other workers, Adolph (1931) and Garside (1966) have shown that amphibians and fish develop more rapidly, and to a smaller size when reared at elevated temperatures. In this study, the aberrant response of growth to temperature is pronounced at both the cooler (20 C) and warmer (30 C) regimes. Not only were smallest specimens recovered from the warmest temperature, and the largest specimens recovered from the coolest temperature, but the body

size of transformees at all three thermal regimes generally increased in time (Fig. 15). Increased body size among later transforming larvae probably reflects the increased length of the feeding period which is under endocrine control.

In crowded conditions, growth among larval anurans has been shown to be reduced by the presence of a variety of intraspecific and interspecific inhibitors (Akin, 1966; Licht, 1967; Richards, 1958, 1962; and Rose, 1960). Although the constant filtering of water with both outside and undergravel filters, and the daily removal of old spinach and feces were designed to have obviated this hazard, it is possible that the larvae were subject to the influence of an intraspecific regulator of density and development. None of the algae cells described by Richards (1958) were observed in the fecal matter of ten larvae that demonstrated inhibited development (>15 months) at 20 C.

The density of larvae maintained (originally 100 specimens per tank) was probably greater than the optimum number for the volume of water. Amstislavskaya (1971) discussed retarded growth and accelerated differentiation rates among <u>Rana terrestris</u> maintained in crowded conditions. The inhibition of some larvae by larger, healthier ones until the large ones transformed, would partially explain why the development time was so greatly lengthened, and why relatively few numbers of larvae transformed each month at 20 C. After the more rapidly developing larvae transformed, the larvae heretofore suppressed (affected by the metabolite) would have opportunity to develop and yet suppress development in other, smaller larvae present. Should inhibitory substances

have been involved, they might have reacted synergistically with endocrine factors that regulate the onset of metamorphosis. Continuous filtration of water by both undergravel and outside filters in the study, however, probably precluded inhibition by anti-metabolites. Conditions of low temperature and abundant food may confer ecological advantages that are detected by biological feed-back mechanisms and result in prolongation of the larval stage (Wilbur and Collins, 1973). Larvae of the genus <u>Rana</u> are extremely variable in the extent of larval development. <u>Rana clamitans</u> may require from 70 to 360 days for transformation (Martof, 1956); <u>R. catesbeiana</u> may require less than one or three years (Dent, 1968; Willis <u>et al.</u>, 1956).

The increased body sizes among larvae in the final samples may represent growing specimens whose development had been inhibited for some time (perhaps weeks), but finally were allowed to complete development when the suppressive, antibiotic producing larvae were lost from the population. In amphibians, the larval stage is most characterized by growth and feeding activity. Whatever the causes of extended development at 20 C, the reason for increasing body sizes in time is likely the result of continued feeding, thus growth, during this time.

The numbers of transformees from each of the 12 replicate tanks did not differ significantly from one another. The frequency observed (Table 29) was not greater than might be expected if the actual frequencies were identical ($\underline{i} \ \underline{e}$. 57 larvae from each tank; $X^2 = 18.63$, P > 0.05). Minor differences of sample sizes among replicate tanks, therefore, are attributable to randomness.

As already noted, maximum body growth is attained by developmental stage 42. Thereafter, the tail is continually resorbed but the body length does not change (Etkin, 1964). Since the transforming larvae removed were past the growth stages, body lengths among stages 42 to 46 represent comparably accurate measures of total body growth at metamorphosis. At these stages the body and degenerating tail can be readily distinguished by a difference in pigmentation.

Considerable variation occurred among mean body lengths of larvae from replicate aquaria at each thermal regime. When tested with the Kruskal-Wallis nonparametric analysis of variance (Sokal and Rohlf, 1969), only the mean lengths of larvae reared in replicate tanks at 30 C were not significantly different among themselves. Mean body lengths among replicate tanks at 20 C and 25 C differed significantly among both groups (Table 29). Differences in body lengths, therefore, did not follow a trend consistent with the positioning of the two separate egg clutches (tanks A and B, C and D). By comparison, however, the differences among mean body length at three temperatures was <u>highly</u> significant (Table 30). Although the "within group" variances are different, "between group" variances are greater by more than 20 orders of magnitude.

The mean body lengths of larvae reared at 25 C and 30 C were more similar to each other than either were to those reared at 20 C. This similarity is evident both in the body lengths and the transformation rates. Rather than demonstrating thermal stress, these data probably represent the influence of sustained, unusually cool temperature in the group reared at 20 C. Both of the warmer temperatures

more closely resemble the thermal ranges commonly encountered by larval Rana pipiens in South Carolina.

Differences in mean total lengths and variances at each tank are affected by the confounding effect of combining data from larvae of different stages. Unlike body lengths, tail lengths (thus total lengths) of larvae are highly correlated to the stage of development (Table 36). Differences among stages caused by variable tail lengths confound those attributable to temperature.

The coefficients of variation might be interpreted as indices of stress. The intermediate thermal regime (25 C) experienced the lowest variance and probably represented the most nearly "optimum" of the three temperatures. Greater coefficients of variations at 20 C and 30 C also reflect the stresses incurred there: temperatures approaching the maximum thermal tolerance at 30 C and severely inhibited development at 20 C (Table 31). The range of body lengths is greater for larvae reared at 20 C and 30 C (18 mm; Table 31), than those at 25 C (15 mm). In addition, the variances at 20 C and 30 C are considerably greater than that at 25 C. These differences probably represent the depressed development of larvae at a constantly reduced temperature (20 C). The low variances of lengths recorded at 25 C may well represent near-optimum development of larvae there. The maximum disparity of values recorded at 20 C and 30 C indicate the aberrant patterns of development at those temperatures.

Mean body lengths of larvae transforming monthly show that the ranges of minimum and maximum values at each thermal regime were from

6 to 8 mm (Table 31). Unlike the other two "populations," larvae transforming at 20 C increased steadily in time both in mean body lengths monthly and maximum length attained (40 mm). Although the mean body lengths of larvae reared at 25 C increased monthly, the maximum body size (34 mm) was attained earlier, in the fifth of approximately eight months. Larvae at 30 C also increased in mean body length monthly, attaining the maximum value (33 mm) in the third of six months. Both variances and coefficients of variations for mean lengths are least at 25 C, further substantiating the favorability of this thermal regime in comparison to the others.

Transformation and Survivorship

Transformation (survivorship excluding mortality) at 25 C and 30 C differs markedly from that at 20 C in rate and form (Fig. 16). All specimens at 25 C had metamorphosed by April; those at 30 C probably would have transformed in March or April. Specimens at 20 C, however, would have taken considerably longer (more than 10 additional months).

When data (n = 302) for "natural" mortality (not accidental, experimental) are added to the numbers of transformations, all curves are smoothed-out (Fig. 17, N = 985). The resulting survivorship curves at 25 C and 30 C are more similar than the transformation rates indicate. After the initial few months, those two groups represent virtually identical trends displaced by a time period of approximately one month. Data for 20 C follows a slower, more consistent rate of decrease. Survivorship at 30 C demonstrated the most rapid decline but closely

resembled that at 25 C. The transformation curves (survivorship excluding mortality) for the 25 C and 30 C series resemble a geometric progression, and the curve for larvae maintained at 20 C more closely approximates an arithmetic (additive) progression, being more linear in appearance.

Although the groups reared at 20 C and 30 C were both terminated prematurely, an approximation of the probable overall survivorship can be estimated by extrapolating the curves to zero. When this is done, the termination dates are March 1972, for the group at 30 C and June 1973, for the group at 20 C. If these termination dates are used to compare survivorship at the three thermal regimes, all larvae would have died within 88 weeks at 20 C, 28 weeks at 25 C and 24 weeks at 30 C. Data are plotted with the assumption that all larvae living at the time would eventually complete metamorphosis. Rather than comparing the groups at the estimated times of zero survivorship, actual values are compared at specific intervals (Table 32).

When one calculates the time intervals required to reach successive survivorship intervals (90%, 75%, 50%, 25%, 10%), the survivorship of larvae at 25 C and 30 C are again found to be quite similar both in the amount of time between levels and their respective proportions. The amount of time (weeks) required for each group to reach the next arbitrary level of survivorship are comparable. Again the series reared at 20 C is shown to be distinctly different, requiring much more time, and in different proportions than the other two thermal regimes. The vast differences between the rates of survivorship and

transformation at 25 C and 30 C as opposed to 20 C are obvious. More detailed quantification and elaborate comparison of these values probably would not be meaningful.

Larvae reared at 20 C differ markedly from the other groups. These data suggest that somewhere between 20 C and 25 C lies a constant temperature below which "normal" development is greatly retarded. The response observed could represent a laboratory effect. Constant thermal regimes, although necessary in quantifying and comparing rate functions and standardizing Paboratory conditions, are not commonly encountered in nature. In thermal ranges where temperatures inhibit the rates of biochemical processes, natural responses (growth, transformation) may be severely distorted. Plasticity inherent in amphibian biology is highly variable even outside thermal considerations. Body weight and time of metamorphosis naturally demonstrate much variability (Adolph, 1931; Brockelman, 1969; Martof, 1956; Pollister and Moore, 1937; and Wilbur, 1972).

Percent Survivorship

A comparison of survivorship among thermal regimes is made in regards to the percent of accountable surviving specimens as a percent of the time that animals were alive in each group (Fig. 18). Such a comparison demonstrates the overall survivorship patterns held to a constant time reference. Actual time periods at different thermal regimes vary from nine days at 35 C to >17 months at 20 C. If the patterns of survivorship were consistent without regards to temperature, or if survivorship varied greatly with temperature, these relationships

should be apparent; they are not. Since the larvae reared at 20 C and 30 C were terminated, the percent of larvae surviving at termination are indicated at 100% total time. In this respect, all curves are not strictly comparable; they might have been slightly different had the larvae at 20 C and 30 C fully terminated. Since so few specimens were left at termination (10% at 20 C and 4% at 30 C), differences are shown to be small in comparison to the total number; corresponding changes in curves are therefore considered negligible.

Whereas no larvae transformed at 35 C, the curve for this group represents mortality only. This fact may account for the disparity exhibited between specimens at this temperature and the other three. At temperatures where transformation occurred (20 C, 25 C, 30 C), the survivorship curves closely approximate each other. The slope of the survivorship curve for larvae at 20 C was the most consistent. The slower, regular pattern contrasts with the more sigmoid patterns at 25 C and 30 C. Because of variance heterogeneity among data, the mathematical values of slopes are not compared. The gradually declining survivorship at 20 C reflects the inhibited development rates that are concommitant with a longer period of growth (Wilbur and Collins, 1973).

As data from 35 C represent mortality only, the curve might not be expected to demonstrate a trend similar, to those at the other thermal regimes, where a majority of data represent transformed larvae. It is included in the interest of completion. Fifty percent of the embryos died in 76% of the time (7 days) and 50% in the final two days. Heavy mortality in two days probably represents one of the more thermally

sensitive stages that embryos had attained. Larval amphibians are known to be extremely sensitive to temperatures changes in early cleavage and gastrulation (Atlas, 1935; Grainger, 1959; King, 1903; Moore, 1942b; Volpe, 1953). The remaining specimens apparently reached another sensitive stage and suffered thermal death in the final two days. Another possibility is that larvae demonstrate only limited tolerance to temperatures as high as 35 C. Extended exposure probably results in greater mortality. Briggs (1947) reports 12% survival among larval <u>R. pipiens</u> subjected to 37 C for a period of four minutes.

Survivorship at all three temperatures declines gradually and steadily for the first 30% of the time (Fig. 18). Thereafter the rates at all three temperatures decline more rapidly. At this time, survivorship is 84% of the larvae at 20 C and 95% of the larvae at 25 C and 30 C. For the first 30% of development time required for premetamorphic development, therefore, specimens die at a fairly constant rate at all temperatures. The significance of this phenomenon (if any) is not clear. Larvae begin to transform soon thereafter and survivorship curves reflect metamorphosis in addition to mortality. Division of these data into components (mortality and transformation), verify that the mortality rates were steady and that differences among temperatures represent the greater numbers of larvae transforming.

Similarity between populations reared at 25 C and 30 C is reiterated in initial and subsequent survivorship patterns. The curve for larvae at 20 C does not represent a massive transformation in short time, but gradual loss of larvae throughout (Fig. 18).
Because of the complex life pattern of <u>R</u>. <u>pipiens</u>, it is difficult to present survivorship data for the organism from embryo to adult. Only the larval stage is aquatic and, thus, commonly subjected to thermal stress. For this reason, the larval stage is treated separately here. Metamorphosis results in the loss of specimens from larval populations and the aquatic habitat as well. Thus, transformation is equated with mortality. Such an interpretation is appropriate for studies focusing on aquatic larval forms that emigrate from water upon metamorphosis.

Growth in Body Size

Body lengths have been shown to be inversely related to the temperature at which specimens are reared: 20 C>25 C>30 C (Table 30). Since transforming larvae were removed or died at several developmental stages, a valid comparison of lengths must be made among specific stages. The trend demonstrated by body lengths of larvae reared at specific stages follows the same pattern as overall data at each temperature (Fig. 19, Table 33). As sample variances are heterogeneous, the ranges of the standard errors do not necessarily indicate statistically significant differences. Trends, nonetheless are consistent and are shown to be significantly different when subjected to the Kruskal-Wallis nonparametric analysis of variance. From Table 33 it can be seen that most specimens removed from each temperature belong to the same developmental stage, and are thus comparable. As already mentioned above ("Reservoir sampling of larval <u>B</u>. <u>terrestris</u>"), this conclusion conforms to those of other workers (Atlas, 1935; Etkin, 1955;

Herreid and Kinney, 1967; Johnson, 1970; Kokurewicz, 1969; Krough, 1914; Mc Laren, 1965; Muto, 1969a, 1972; Parker, 1965; Purcell, 1968; Rugh, 1951; Ryan, 1941). Growth inhibition is directly related to faster developmental rates. Both responses are characteristic of environments at higher temperatures. Wilbur and Collins (1973) have described the relationship of growth and development parameters to formulate an ecological model of amphibian metamorphosis.

Abnormalities

Major abnormalities encountered in larvae included crooked spine, paralyzed hindlimb, and edemaceous or emaciated body condition. All of these anomalies appeared to some degree in all three thermal regimes where metamorphosis occurred.

Edemaceous and emaciated body conditions were difficult to determine and accurately quantify, and will not be compared. These two designations were necessarily more subjective than the presence or absence of a paralyzed hindlimb or crooked spine. Although specimens in each group were affected to some extent, edemaceous larvae were more apparent at 20 C and emaciated larvae at 25 C and 30 C. Specimens at 30 C were severely affected by crooked spines and paralyzed hindlimbs. Data are presented for larvae demonstrating a crooked spine or paralyzed hindlimb.

Crooked Spine

Specimens with crooked spines were affected to various degrees of severity. Some larvae possessed a slight curve in the basal part of

the tail; severely affected specimens had tails convoluted more than 360°. Pelvic girdles of these specimens may have been deleterously affected, as distorted backs remained after metamorphosis was complete. The permanent curve in the tail of the larvae impaired swimming motion, to the degree that the spine was bent. Severely afflicted specimens were capable only of circular swimming; slightly affected specimens demonstrated awkward, irregular, helical swimming motion (Fig. 20). The occurrence of high numbers of specimens with crooked spines at 30 C is interpreted as an indication of stress incurred there.

Mechanisms involved in the appearance of crooked spine in larvae at the three thermal regimes are not fully understood. Several workers have related its occurrence in some stocks of experimental laboratory cultured R. pipiens (Jane Kaltenback, George Nace, personal communication). Others have published studies with illustrations of larvae demonstrating this same phenomenon (Dent, 1968; Etkin, 1964; Gordon et al., 1945). Very little appears to be known about its occurrence or the influence of external factors on this phenomenon. Moore (1942) observed laboratory reared, larval R. pipiens with "crooked tails." He attributed the occurrence of this abnormality (and others) to elevated temperatures. It seems that the condition does not appear until advanced stages of metamorphosis; affected specimens in this study were not recorded prior to stage 41. The presence of specimens with crooked spines during climax metamorphosis may indicate that this phenomenon is somehow related to tail resorption preceding transformation.

Crooked spines occurred in 170 (74%) of the larvae transforming at 30 C, 34 (10%) of the larvae transforming at 25 C and 43 (21%) of the larvae transforming at 20 C (Fig. 20, Table 34). The unlikely occurrence of affected specimens appearing in the proportions reported indicate that some mechanism is involved other than random variation $(X^2 = 138.4, P < 0.005)$. These data show that the appearance of crooked spines is partially influenced by heat. The highest proportion of affected larvae occurred at the highest temperature: 30 C. The lowest proportion, however, occurred at the intermediate temperature: 25 C. The fact that some larvae at all three thermal regimes possessed crooked spines suggests that although its demonstration is thermally influenced, its propensity is doubtless genetic. Low levels (10-21%) might be expected to occur exclusive of thermal influence, and probably represent inherent variability. Some of the larvae demonstrate this anomaly at any temperature, but elevated temperature increases the likelihood of its occurrence. It is interesting to note that the hundreds of larval Rana pipiens sampled in the field studies never included a single specimen affected with a crooked spine. The absence of such affected larvae probably attests to negative natural selection pressures exerted upon these organisms in nature. A further inquiry into the mechanisms involved await histological analysis of specimens.

When the mean body lengths of specimens are compared at each temperature, there is no apparent difference between affected and unaffected larvae (Table 35). Stage by stage analysis of body lengths at the three temperatures also reveals that body lengths differ

according to thermal regime only. Values for larvae with normal and crooked spines are clearly comparable at all stages at each temperature except perhaps for stages 44 and 45 (Table 36). These differences, however, are not considered significant and they follow no distinct trend. Mean tail lengths differ at specific stages and temperatures, but not in a consistent manner. The variance of these comparisons were generally greater in specimens with a crooked spine. This parameter was measured to detect possible differences in tail resorption rates; no distinct trends were apparent.

Gordon <u>et al</u>. (1945) reported the occurrence of "peculiar tail abnormalities characterized by a twisting confined to the basal region" in experimental <u>R</u>. <u>pipiens</u>. The phenomenon was observed in most thiourea treated specimens and rarely in untreated controls. Radiographic and histological studies demonstrated that these tails reveal no abnormal skeletal growth but rather an overgrowth of connective tissue.

Muto and Hasegawa (1969) reported that larvae of the frog <u>Rana</u> <u>japonica</u> cultured at temperatures "higher than normal" demonstrated wavy tails that closely resemble those described herein. They showed that no wavy tails developed in larvae reared at room temperature or at 20 C, although some of the larvae reared at 25 C and all of those at 30 C possessed such abnormalities. Histological studies of Muto and Hasegawa (1969) and those of Gordon <u>et al</u>. (1945) revealed that the wavy tails represented a winding of the notochord as it increased in length more rapidly than the surrounding muscle tissues. Unlike Muto and Hasegawa's

data, abnormally developed tails in laboratory reared <u>R</u>. <u>pipiens</u> occurred at all temperatures to some extent. The absence of affected specimens at lower temperatures in Muto and Hasegawa's study suggests that the occurrence of wavy tails is environmental rather than genetic.

Curved tails have also been shown to occur in embryonic herring subjected to high temperature (Kudinskii, 1969). The morphological structure causing the abnormality in this case was not defined.

Paralyzed Hindlimbs

To a lesser extent, the frequency of paralyzed hindlimbs was related to temperature. Although only 17 (2.5%) of the 683 transformed larvae demonstrated this anomaly, a chi-square analysis revealed that the frequency of occurrence was significantly nonrandom among temperatures. Since the proportion of affected larvae was apparently associated with the level of thermal influence (Table 34), it is interpreted as a thermal response. The occurrence of paralyzed hindlimbs is apparently a more subtle thermal response than crooked spine. Data for paralyzed hindlimbs are considered insufficient to reveal specific conclusions.

Muto (1969a, 1969b, 1970a, 1970b) has reported heat induced malformations in the skeleton (hindlimbs and forelimbs) of <u>Bufo vulgaris</u> reared at 30 C. Edema and retarded development were observed at 30 C (Muto, 1969a). Such anomalous development patterns were not observed in <u>R</u>. <u>pipiens</u> in this study.

Crooked spines are shown to be strongly related to thermal loading, and paralyzed hindlimbs significantly so. Both of these anomalies inflict obvious disadvantages on affected larvae. One might not expect to encounter such clearly disadvantageous traits in large numbers; selection against such nonadaptive characters would likely keep them in low numbers. Their ecological significances, therefore, might be quite far reaching. Among several hundreds of larval Rana pipiens removed from heated areas in the heated reservoir, never were any specimens seen or collected that demonstrated either of these maladies. This might suggest that these anomalies are responses to constant (laboratory) temperature, and thus not likely to be observed in nature. The rather common occurrence of this trait among laboratory populations, however, suggests that it should be expected in field situations as well. Another possible interpretation is that affected larvae succumb to selective forces (i e. eaten by prey) and simply may not survive long enough to be collected.

SUMMARY AND CONCLUSIONS

Pitfall Trapping Survey

1. Of 13 anuran species trapped in pitfalls peripheral to Pond C Reservoir, three dominant species (<u>R. pipiens, B. terrestris</u>, and <u>G. carolinensis</u>) exhibited massive yearly migrations of breeding adults and emergent young.

2. Emergent young of <u>B</u>. <u>terrestris</u> and <u>G</u>. <u>carolinensis</u> were trapped along the reservoir during the same months that they were trapped elsewhere on the Savannah River Plant. Emerging <u>R</u>. <u>pipiens</u>, however, were not restricted to seasonal occurrence characteristic of unheated areas. Recently transformed specimens were encountered whenever larvae were trapped (during 11 months of the year). Breeding patterns are apparently modified by the increased temperatures at the reservoir.

3. Captures of <u>R</u>. <u>pipiens</u> in pitfalls along the reservoir indicate that adults do breed and larvae develop in certain parts of the heated reservoir.

4. Although considerable development of larval amphibians occurs in cool seepage ponds flanking the reservoir, the numbers of <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> encountered reflect the severity of the thermal gradient along the reservoir's cool arm.

Reservoir Sampling with Dipnets and Minnow Traps

1. Continued sampling of larvae in the vicinity of Pond C Reservoir confirms that larval anurans of five species were found to develop in the heated waters.

2. Abundance, species diversity and density of larval anuran populations generally reflected the level of thermal loading sustained.

3. Egg and embryonic mortality was severe in areas receiving the greatest amounts of thermal loading, and negligible in cool seepage ponds peripheral to the reservoir.

4. Within the ranges of thermal tolerance, the lengths of specimens among heated and cool microhabitats varied inversely with the degree of thermal loading.

Reservoir Sampling of Larval Bufo terrestris

1. The cooling effect of shoreline seepage allows the Southern toad (<u>B. terrestris</u>) to successfully breed and develop in restricted parts of Pond C Reservoir receiving lethal levels of thermal loading.

2. Heavy mortality of toad eggs and embryos occurs in the heated reservoir. Within the ranges of thermal tolerance, however, rates of larval growth and development differ in proportion to the level of thermal loading. Larval <u>B. terrestris</u> grow to a larger size but develop more slowly at cooler temperatures.

3. Constantly elevated temperatures in the reservoir prevent anurans from benefiting from reduced thermal levels naturally associated with nocturnal breeding.

Laboratory Rearing of Larval Rana pipiens

1. Embryonic <u>R</u>. <u>pipiens</u> reared at a constant temperature regime of 35 C suffered total mortality within nine days.

2. Within the limits of thermal tolerance, larvae reared at warmer temperatures (30 C and 25 C) developed more rapidly but grew less than those reared at the coolest temperature (20 C), where larvae demonstrated markedly inhibited rates of development.

3. The occurrence of abnormalities (crooked spine and paralyzed hindlimbs) was greater at 30 C than 20 C or 25 C.

4. Even aquatic ecosystems receiving lethal levels of thermal loading may contain habitable microenvironments created by local seepage or other physical conditions.

5. Anurans are shown to deposit eggs in the heated reservoir at lethal or near-lethal temperatures, where mortality may be very high (90%).

6. Laboratory rearing and field sampling of larval <u>R</u>. <u>pipiens</u> and <u>B</u>. <u>terrestris</u> all corroborate that growth is less, and development greater in heated as opposed to unheated ecosystems.

7. Although no affected specimens were observed in the field conditions, the occurrence of abnormally developed spines and hindlimbs among laboratory reared <u>R</u>. <u>pipiens</u> was greatest at the higher temperature. The absence of these abnormalities in nature is interpreted as a manifestation of negative natural selection pressures exerted there.

LITERATURE CITED

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LITERATURE CITED

- Adolph, Edward F. 1931. Body size as a factor in the metamorphosis of tadpoles. Biol. Bull. (Woods Hole) 61:376-386.
- Akin, Gwynn C. 1966. Self-inhibition of growth in <u>Rana pipiens</u> tadpoles. Physiol. Zool. 39:341-356.
- Alabaster, J. S. 1964. The effect of heated effluents on fish. Adv. Water Pollut. 1:261-292.
- Altig, Ronald G. 1970. A key to the tadpoles of the continental United States and Canada. Herpetologica 26:180-207.
- Amstislavskaya, T. S. 1971. The effect of growth and development regulators on tissue growth in amphibian larvae. Sov. J. Ecol. 2:555-556 (Engl. transl. Ekologiya).
- Atlas, Meyer. 1935. The effect of temperature on the development of <u>Rana pipiens</u>. Physiol. Zool. 8:290-310.
- Bachmann, Konrad. 1969. Temperature adaptations of amphibian embryos. Am. Nat. 103:115-130.
- Ballinger, Royce E., and Charles O. McKinney. 1966. Developmental temperature tolerance of certain anuran species. J. Exp. Zool. 161:21-28.
- Bèlehrádek, Jan. 1935. Temperature and living matter. Gebrüder Borntraeger, Berlin. 277 pp.
- Brattstrom, Bayard H. 1962. Thermal control of aggregation behavior in tadpoles. Herpetologica 18:38-46.
- Brattstrom, Bayard H. 1963. A preliminary review of the thermal requirements of amphibians. Ecology 44:238-255.
- Braun, E. Lucy. 1964. Deciduous forests of eastern North America. Hafner Publishing Co., New York, 596 pp.
- Brett, J. R. 1956. Some principles in the thermal requirements of fishes. Q. Rev. Biol. 31:75-87.

- Briggs, Robert. 1947. The experimental production and development of triploid frog embryos. J. Exp. Zool. 106:237-266.
- Brock, Thomas D. 1970. High temperature systems. Ann. Rev. Ecol. Syst. 1:191-220.
- Brockelman, Warren Y. 1969. An analysis of density effects and predation in Bufo americanus tadpoles. Ecology 50:632-644.
- Brown, Herbert A. 1967a. Embryonic temperature adaptations and genetic compatibility in two allopatric populations of the spadefoot toad, <u>Scaphiopus</u> hammondii. Evolution 21:742-761.
- Brown, Herbert A. 1967b. High temperature tolerance of the eggs of a desert anuran, <u>Scaphiopus</u> hammondii. Copeia 1967:365-370.
- Cairns, John, Jr. 1972. Coping with heated waste water discharges from steam-electric power plants. Bioscience 22:411-419.
- Clark, John R. 1969. Thermal pollution and aquatic life. Sci. Am. 220 (3):18-27.
- Coutant, Charles C. 1968. Thermal pollution--biological effects. A review of the literature of 1967. J. Water Pollut. Control Fed. 40:1047-1052.
- Coutant, Charles C. 1969. Thermal pollution--biological effects. A review of the literature of 1968. J. Water Pollut. Control Fed. 41:1036-1053.
- Coutant, Charles C. 1970. Thermal pollution--biological effects. A review of the literature of 1969. J. Water Pollut. Control Fed. 42:1025-1057.
- Coutant, Charles C. 1971. Thermal pollution--biological effects. A review of the literature of 1970. J. Water Pollut. Control Fed. 43:1292-1334.
- Crozier, W. J. 1926. On curves of growth, especially in relation to temperature. J. Gen. Physiol. 10:53-73.
- Das, Asit B., and C. Ladd Prosser. 1967. Biochemical changes in tissues of goldfish acclimated to high and low temperatures. I. Protein synthesis. Comp. Biochem. Physiol. 21:449-467.
- Davenport, C. B., and W. E. Castle. 1895. Studies on morphogenesis. III. On the acclimation of organisms to high temperature. Arch. Entwicklugsmech. Org. 2:227-247.

- Dent, James N. 1968. Survey of amphibian metamorphosis. Pages 271-311 in metamorphosis, a problem in developmental biology. Edited by William Etkin and Lawrence I. Gilbert. Appleton-Century-Crofts, New York, 459 pp.
- de Vlaming, Victor L., and R. Bruce Bury. 1970. Thermal selection in tadpoles of the tailed-frog, <u>Ascaphus</u> truei. J. Herpetol. 4:179-189.
- Dunlap, Donald G. 1968. Critical thermal maximum as a function of temperature of acclimation in two species of hylid frogs. Physiol. Zool. 41:432-439.
- Etkin, William. 1955. Metamorphosis. Pages 631-663 in Analysis of development. Edited by B. H. Willier, P. A. Weiss, and V. Hamburger. Saunders, Philadelphia, 735 pp.
- Etkin, William. 1964. Metamorphosis. Pages 427-468 in Physiology of the Amphibia. Edited by J. A. Moore. Academic Press, New York, 654 pp.
- Etkin, William. 1968. Hormonal control of amphibian metamorphosis. Pages 313-348 in Metamorphosis, a problem in developmental biology. Edited by William Etkin and Lawrence Gilbert. Appleton-Century-Crofts, New York, 459 pp.
- Fry, F. E. J. 1967. Responses of vertebrate poikilotherms to temperature. Pages 375-409 in Thermobiology. Edited by A. H. Rose. Academic Press, New York, 653 pp.
- Garside, E. T. 1966. Effects of oxygen in relation to temperature on the development of embryos of brook trout and rainbow trout. J. Fish. Res. Board Canada 23:1121-1134.
- Gibbons, J. Whitfield, and David H. Bennett. 1974. Determination of anuran terrestrial activity patterns by a drift fence method. Copeia 1974:236-243.
- Gibbons, J. Whitfield, and Rebecca R. Sharitz. 1974. Thermal Ecology. US AEC Symposium series (conf. 730505) (in press).
- Gordon, Albert S., E. D. Goldsmith, and H. A. Chaparipper. 1945. The effects of thiourea on amphibian development. Growth 9:19-41.
- Gosner, Kenneth L. 1960. A simplified table for staging anuran embryos and larvae, with notes on identification. Herpetologica 16:183-190.
- Grainger, J. N. R. 1959. The effects of constant and varying temperatures on the developing eggs of <u>Rana</u> temporaria L. Zool. Anz. 163:267-277.

- Hathaway, Edward S. 1928. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. Bull. U.S. Bureau of Fish. 3:169-192.
- Heilbrunn, Lewis V. 1956. An outline of general physiology. 3rd ed. W. B. Saunders Co., Philadelphia, 818 pp.
- Herreid, Clyde F., and Stephen Kinney. 1967. Temperature and development of the wood frog, <u>Rana sylvatica</u>, in Alaska. Ecology 48:579-590.
- Hoadley, Leigh. 1938. The effect of supramaximum temperatures on the development of Rana pipiens. Growth 2:25-48.
- Hoar, William S. 1966. General and comparative physiology. Prentice-Hall, Englewood Cliffs, N.J., 815 pp.
- Hutchison, Victor H. 1961. Critical thermal maxima in salamanders. Physiol. Zool. 34:92-125.
- Johnson, Clifford R. 1970. Observations on body temperatures, critical thermal maxima and tolerance to water loss in the Australian hylid, Hyla caerula (White). Proc. R. Soc. Queensl. 82:47-50.
- Jones, John R. 1964. Fish and river pollution. Butterworths, London, 203 pp.
- Kennedy, V. S., and J. A. Mihursky. 1967. Bibliography on the effects of temperature in the aquatic environment. Contrib. #326, Dept. of Water Resources, Md., 89 pp.
- King, Helen D. 1903. The effects of heat on the development of the toad's egg. Biol. Bull. (Woods Hole) 5:218-232.
- Kokurewicz, Boguslaw. 1969. The influence of temperature on the embryonic development of the perches: <u>Perca fluviatilis</u> L. and Lucioperca lucioperca (L.). Zool. Pol. 19:47-67.
- Krenkel, Peter A., and Frank L. Parker (eds.). 1969. Biological aspects of thermal pollution. Vanderbilt Univ. Press, Nashville, Tenn., 407 pp.
- Kroger, Richard L. 1973. Biological effects of fluctuating water levels in the Snake River, Grand Teton National Park, Wyoming. Amer. Midl. Natur. 89:478-481.
- Krogh, August. 1914. On the influence of the temperature on the rate of embryonic development. Z. Allg. Physiol. 16:163-177.

- Kudinskii, O. Yu. 1969. Embryonic development of "small" White Sea herring (<u>Clupea</u> <u>harengus</u> <u>pallasi</u> n. maris-albi var β) in relation to temperature. Dokl. Akad. Nauk. SSSR. Biol. Sci. Sect. (Engl. transl.).
- Levin, Arthur A., <u>et al.</u> 1970. A comprehensive appraisal of the effects of cooling water discharge on aquatic ecosystems. Presented at the Atomic Industrial Forum, Inc. Public Affairs Workshop "Why Nuclear Power?" Hilton Head Island, S.C. (Sept. 13-16, 1970). Battelle Memorial Inst., Columbus Laboratories, 505 King Ave., Columbus, Ohio 43201.
- Levin, Arthur A., <u>et al</u>. 1972. Thermal discharges: ecological effects. Environ. Sci. Technol. 6:224-229.
- Licht, Lawrence E. 1967. Growth inhibition in crowded tadpoles: intraspecific and interspecific effects. Ecology 48:736-745.
- Licht, Lawrence E. 1971. Breeding habits and embryonic thermal requirements of the frogs, <u>Rana aurora aurora and Rana pretiosa</u> pretiosa, in the Pacific Northwest. Ecology 52:116-124.
- Lillie, Frank R., and R. P. Knowlton. 1897. On the effects of temperature on the development of animals. Zool. Bull. 1:179-193.
- Limbaugh, Beverly A., and E. Peter Volpe. 1957. Early development of the Gulf Coast toad, <u>Bufo</u> valliceps Wiegmann. Am. Mus. Novit. 1842:1-32.
- Martof, Bernard. 1956. Growth and development of the green frog, <u>Rana clamitans</u>, under natural conditions. Am. Midl. Nat. 55:101-117.
- Mc Laren, Ian A. 1965. Temperature and frog eggs. J. Gen. Physiol. 48:1071-1079.
- Merriman, Daniel. 1970. The calefaction of a river. Sci. Am. 222 (5):42-52.
- Mihursky, J. A. 1969. Patuxent thermal studies: summary and recommendations. Univ. Md. Nat. Resour. Inst., Spec. Rept. No. 1, 20 pp.
- Mihursky, J. A., and V. S. Kennedy. 1967. Water temperature criteria to protect aquatic life. Am. Fish. Soc. Spec. Publ. 4:20-32.
- Moore, John A. 1939. Temperature tolerance and rates of development in the eggs of Amphibia. Ecology 20:459-478.

- Moore, John A. 1942a. The role of temperature in speciation of frogs. Biol. Symp. 6:189-213.
- Moore, John A. 1942b. Embryonic temperature tolerance and rate of development in <u>Rana catesbeiana</u>. Biol. Bull. (Woods Hole) 83: 375-388.
- Moore, John A. 1949. Geographic variation of adaptive characters in Rana pipiens Schreber. Evolution 3:1-24.
- Moore, John A. (ed.). 1964. Physiology of the Amphibia. Academic Press, New York, 654 pp.
- Muto, Yoshinobu. 1969a. Hindlimb development and malformations of toes in the larvae reared at high temperature in the toad, <u>Bufo</u><u>vulgaris formosus</u>. Congenital Anomalies 9:1-12.
- Muto, Yoshinobu. 1969b. Anomalies in the hindlimb skeletons of the toad larvae reared at high temperature. Congenital Anomalies 9:61-73.
- Muto, Yoshinobu. 1970a. Development of the hindlimb skeleton and its anomalies induced by cultivation at high temperature in the toad larvae. Bull. Aichi. Univ. Educ. 19:43-53.
- Muto, Yoshinobu. 1970b. Digital malformations in the forelimbs of the toad reared at high temperature. Congenital Anomalies. 10:135-147.
- Muto, Yoshinobu. 1972. Change in developmental pattern in the toad larva at different temperatures. Bull. Aichi. Univ. Educ. 21:111-123.
- Muto, Yoshinobu, and Hitoshi Hasegawa. 1969. Wavy tail of the larva of <u>Rana japonica</u> raised at high temperature. Bull. Aichi. Gakugei Univ. 9:147-154.
- Nelkin, Dorothy. 1971. Nuclear power and its critics, the Cayuga Lake controversy. Cornell Univ. Press, Ithaca, N.Y., 128 pp.
- Odum, Eugene P. 1971. Fundamentals of Ecology. 3rd ed. W. B. Saunders, Philadelphia, 574 pp.
- Packard, Gary C. 1972. Evolutionary compensation for temperature: oxygen consumption <u>in vitro</u> of tissues from the toads <u>Bufo boreas</u> and <u>Bufo woodhousei</u>. Physiol. Zool. 45:310-315.
- Parker, Gary C. 1965. The influence of temperature on oxygen consumption in <u>Rana pipiens</u> tadpoles. M.S. thesis, Ball State University, Muncie, Ind., 42 pp.

- Pollister, A. W., and John A. Moore. 1937. Tables for the normal development of <u>Rana sylvatica</u>. Anat. Rec. 68:489-496.
- Precht, Herbert. 1958. Theory of temperature adaptation in cold blooded animals. Pages 50-78 in Physiological Adaptation. Edited by C. Ladd Prosser. Am. Physiol. Soc., Washington, D.C., 185 pp.
- Prosser, C. Ladd (ed.). 1958. Physiological Adaptation. Amer. Physiol. Soc., Washington, D.C., 185 pp.
- Prosser, C. Ladd (ed.). 1967. Molecular mechanisms of temperature adaptations. Publ. 84, Am. Assoc. Adv. Sci., Washington, D.C., 390 pp.
- Prosser, C. Ladd (ed.). 1973. Comparative animal physiology. 3rd. ed. Vol. I. W. B. Saunders, Philadelphia, 456 pp.
- Purcell, Jerry W. 1968. Embryonic temperature adaptations of Southwestern populations of <u>Rana pipiens</u>. M.S. thesis, Texas Technological College, Lubbock, 46 pp.
- Raney, Edward C., and Bruce Menzel. 1969. Heated effluents and effects on aquatic life with emphasis on fishes, a bibliography. Phila. Elec. Co., Cornell Water Res. Mar. Sci. Cent., and Ichthyolog. Assoc. Bull. no. 2, N.Y., 470 pp.
- Richards, Christina M. 1958. The inhibition of growth in crowded <u>Rana</u> pipiens tadpoles. Physiol. Zool. 31:138-151.
- Richards, Christina M. 1962. The control of tadpole growth by algalike cells. Physiol. Zool. 35:285-296.
- Rose, Anthony H. (ed.). 1967. Thermobiology. Academic Press, New York, 653 pp.
- Rose, S. Meryl. 1960. A feedback mechanism of growth control in tadpoles. Ecology 41:188-199.
- Rugh, Roberts. 1951. The frog, its reproduction and development. McGraw-Hill, New York, 336 pp.
- Ruibal, Rodolfo. 1957. An altitudinal and latitudinal cline in <u>Rana</u> <u>pipiens</u>. Copeia 1957:212-221.
- Ruibal, Rodolfo. 1959. The ecology of a brackish water population of <u>Rana pipiens</u>. Copeia 1959:315-322.
- Ruibal, Rodolfo. 1962. The ecology and genetics of a desert population of <u>Rana pipiens</u>. Copeia 1962:189-195.

- Ryan, Francis J. 1941. Temperature change and the subsequent rate of development. J. Exp. Zool. 88:25-54.
- Seibel, Roberta V. 1970. Variables affecting the critical thermal maximum of the leopard frog, <u>Rana pipiens</u> Schreber. Herpetologica 26:208-213.
- Sokal, Robert R., and F. James Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, 776 pp.
- Summers, Claude M. 1971. The conversion of energy. Sci. Am. 224 (3):148-160.
- Tarzwell, Clarence M. 1970. Thermal requirements to protect aquatic life. J. Water Pollut. Control Fed. 42:824-828.
- Trembly, F. J. 1960. Research project on effects of condenser discharge water on aquatic life. Progress report 1956-1959. The Institute of Research, Lehigh Univ., Bethlehem, Pa.
- Troshin, A. S. 1967. The cell and environmental temperature. Pergamon Press, New York, 464 pp.
- Ushakov, V. P. 1972. Instability and conservatism of heat resistance of the organism, cells, and proteins of poikilotherms in the process of temperature acclimation. Proc. Int. Congr. Zool. 17:1-30.
- Vernberg, F. John, and Winona B. Vernberg. 1970. The animal and the environment. Holt, Rinehart and Winston, Inc., New York, 398 pp.
- Volpe, E. Peter. 1953. Embryonic temperature adaptations and relationships in toads. Physiol. Zool. 26:344-354.
- Volpe, E. Peter. 1957a. Embryonic temperature tolerance and rate of development in Bufo valliceps. Physiol. Zool. 30:164-176.
- Volpe, E. Peter. 1957b. The early development of <u>Rana capito sevosa</u>. Tulane Stud. Zool. 5:207-225.
- Whittow, G. C. (ed.). 1970. Comparative physiology of thermoregulation. Vol. I. Academic Press, New York, 162 pp.
- Wilbur, Henry M. 1972. Competition, predation, and the structure of the Ambystoma-Rana sylvatica community. Ecology 53:3-21.
- Wilbur, Henry M., and James P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305-1314.

- Willis, Yuell L., D. L. Moyle, and T. S. Baskett. 1956. Emergence, breeding, hibernation, movements, and transformation of the bullfrog, Rana catesbeiana, in Missouri. Copeia 1956:30-40.
- Wright, Albert H., and Anna A. Wright. 1949. Handbook of frogs and toads. Cornell Univ. Press, Ithaca, New York, 640 pp.
- Zweifel, Richard G. 1957. Studies of the critical thermal maxima of salamanders. Ecology 38:64-69.
- Zweifel, Richard G. 1968. Reproductive biology of anurans of the arid Southwest, with emphasis on adaptation of embryos to temperature. Bull. Amer. Mus. Natur. Hist. 140:1-64.

