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University of Nevada,

Las Vegas

THERMAL ECOLOGY OF THE
PAHRUMP KILLIFISH,
EMPETRICHTHYS LATOS LATOS Miller

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in Biology

by

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May 1977

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INTRODUCTION

The Pahrump killifish, Empetrichthys latos latos Miller, is a cyprinodont fish originally restricted to the Pahrump Valley, Nye County, Nevada. The most characteristic features of this genus are the tubercular-shaped molar teeth and the greatly enlarged upper and lower pharyngeal bones (Miller, 1948). Additionally, the fish has cycloid scales, 29-33 in the lateral series, a protractile premaxillary, dorsal and anal fins placed far back on the body and an absence of pelvic fins (Miller, 1948). Little visible sexual dimorphism is present in E. l. latos although mature adults have differences in coloration with the male being more silvery with a bluish tinge, lacking the black mottled appearance of mature females. Males also tend to be somewhat smaller than females. Both sexes display a yellowish coloration of the fins when in optimal environments.

The species E. latos was described by Miller in 1948 who recognized three distinct subspecies. The type locality for each subspecies is, E. l. latos from Manse Spring, E. l. pahrump from Pahrump Spring, and E. l. concavus from Raycraft Spring (Miller, 1948). An additional species of Empetrichthys which occurred in Ash Meadows adjacent to Pahrump Valley, E. merriami, is now extinct (Minckley and Deacon, 1968).

The genus Eupetrichthys is most closely related to the genus Crenichthys of the White River Basin and Railroad Valley in eastern Nevada, with both genera derived from a common funduline ancestor (Miller, 1948; Miller, 1949; Miller, 1950; Uyeno and Miller, 1967).

During the 1950's the habitats of E. l. pahrump and E. l. concavus were destroyed eliminating those subspecies (Minckley and Deacon, 1968). In Manse Spring E. l. latos suffered a serious decline in numbers in 1962 as a result of the introduction of goldfish, Carassius auratus, and modification of habitat (Deacon, Hubbs, and Zahruanec, 1964; Hubbs and Deacon, 1964; Deacon and Bunnel, 1970). This decline in numbers, along with the limited habitat of the species, resulted in the listing in the Federal Register of E. l. latos as an endangered species (32 FR 4001; March 11, 1967).

E. l. latos was eliminated from Pahrump Valley in August 1975 when Manse Spring failed as a result of excessive pumping of ground water. Today this last surviving subspecies survives only from transplants that were made prior to the drying of Manse Spring. The largest population is in the Corn Creek Pond on the Desert National Wildlife Fange, Clark County, Nevada. My study was primarily concerned with fish from this population.

The Pahrump killifish was originally introduced into Corn Creek Pond in August 1971 when 29 fish were brought from Manse Ranch. By November 1973, the population had increased to approximately 1300 fish. Subsequently, a decline in numbers of killifish occurred until only 165 fish were left when the pond was drained in April 1976. At that time the pond was drained and poisoned to eliminate mosquitofish,

the species responsible for the decline. Following detoxification, killifish were reintroduced into the pond, spawned almost immediately, and young were sighted in July 1976. The population, as estimated by a mark-and-recapture census in November, had grown to approximately 2000 fish.

The transplanted populations of Pahrump killifish have experienced conditions quite unlike those of Manse Spring. In the Manse Spring pool water temperatures were constant at about 24 C, while transplanted fish have experienced low temperatures of 4 C under ice cover in a horse trough at Corn Creek. In the turbid and stagnant water of Latos Pools along the Colorado River, Pahrump killifish have withstood annual temperature fluctuations from below 10.5 C to 25 C for five years.

It was with the knowledge that the Pahrump killifish has temperature tolerances exceeding the environmental temperatures encountered in its native habitat that this study was begun. The investigation was aimed at defining the tolerance of this unique species; in particular, the critical thermal maxima (CTM) of fish at different acclimation temperatures, the oxygen tolerance of the fish, the effects of temperature and oxygen levels upon fish distribution at Corn Creek, and the preferred temperatures of fish from different acclimation temperatures.

Two major procedures have been followed in the determination of temperature tolerances of fishes. The first is determination of the lethal temperature. This procedure involves the removal of acclimated fish to chambers of higher or lower temperature and observation of the fish until death occurs. This point, where

temperature becomes a lethal factor, has been designated as the "incipient lethal level" (Hart, 1947). This procedure has given rise to other terminology such as "zone of tolerance", which is the temperature regime bounded by the upper and lower incipient lethal levels, the "resistance time", which is the amount of time the fish can live at temperatures beyond the incipient lethal levels, and the "ultimate upper and lower incipient levels", which are the points at which further increase or decrease in acclimation temperature fails to produce a change in the incipient lethal level (Hart, 1947). The method of determining lethal temperatures for the fishes has been widely employed on a large number of fish species. Brett (1956) summarized the results of lethal tolerance tests on 23 species of fishes demonstrating a wide diversity in lethal temperatures among different families of fish.

The second method of determining temperature tolerances of fish is the "critical thermal maximum" or "CTMax". This method has been employed to determine the ability of fish to survive for short periods at elevated temperatures. It is determined by increasing the water temperature of acclimated fish at a constant rate until loss of equilibrium or death results. It has been used successfully on species of Cyprinodon (Lowe and Heath, 1969; Otto and Gerking, 1973) as well as on the unarmoured stickleback, Gasterosteus aculeatus williamsoni (Feldmeth and Baskin, 1976). The same procedure can be employed to determine the ability to tolerate low temperatures by decreasing the water temperature until loss of equilibrium or death results. This lower limit is the "critical thermal minimum"

or "CTMin": The method of using CTM to delineate the tolerances of the Pahump killifish was chosen primarily because it was possible to determine the CTM without causing the death of the fish. This allowed experiments within the limitations of the Nevada Department of Fish and Game permit which did not provide for the sacrifice of fish in this type of work.

The fish must become acclimated to a chosen temperature prior to the beginning of any temperature tolerance experiment. The significance of acclimation and the reporting of the acclimation temperature has been discussed by Fry (1947). The acclimation temperature, when increased, raises the CTM and when decreased lowers the CTM, thus, its importance in the analysis of observed thermal limits. Acclimation involves, in part, biochemical adaptation to a new environment. Hochachka and Somero (1973) stated that when an animal encounters adverse conditions from which it cannot escape, its alternative for survival is adaptation to the new conditions. This biochemical adaptation involves, in part, the isoenzyme pattern of liver lactate dehydrogenase, hemoglobin concentrations, and microhematocrit levels in the goldfish, Carassius auratus (Houston and Cyr, 1974; Tsukuda and Ohsawa, 1974).

Time involved in acclimation has been calculated for a number of fish species. Campbell and Davies (1975) showed Blennius pholis to completely acclimate from 10 C to 20 C in two to three weeks on the basis of metabolic rate measurements. Brett (1944) determined that the bullhead, Ictalurus nebulosus, acclimated to 20 C can be fully acclimated to 28 C in as little as 12 hours. The fathead minnow,

Pimephales promelas, acclimated to 24 C requires 22 days to fully acclimate to 16 C. The criteria for determination of full acclimation for I. nebulosus and P. promelas was the point at which increased acclimation time failed to increase lethal limits. In general, acclimation to higher temperatures takes place within a one week period for most fish, while acclimation to low temperature is a slower process requiring three to four weeks.

MATERIALS AND METHODS

Critical thermal limits

Pahrump killifish were acclimated for at least two weeks before testing for CT_{Max} and at least three weeks before testing for CT_{Min}. Acclimation either took place in the field or the laboratory. Hochachka and Somero (1973) make a distinction between laboratory acclimation and acclimation under natural conditions. When adaptation occurs in the lab in response to manipulation it is correctly termed acclimation, however, when several uncontrolled environmental variables i.e. photoperiod, food, competition, are involved as they are under natural conditions the correct terminology is acclimatization. For the purposes of this study these differences were considered to be minor. When in the lab, fish were maintained in 40 liter aquaria equipped with charcoal-glass wool filters. The temperatures for the tanks were kept constant within plus or minus one degree Celcius by 100 watt, thermostatically controlled, aquarium heaters or copper cooling coils immersed in the tanks. Fish not lab acclimated were taken from the constant temperature spring sources at Corn Creek or the UNLV rearing pools on campus where temperatures were monitored continuously. During acclimation of all fishes involved in the study the photoperiod corresponded to natural daylength. Fish in the lab were fed daily with the commercial fish food, TetraMin.

Fish were tested singly initially until it was certain the fish could survive testing. Later tests used up to five fish at one time. Testing took place in a 13.5 liter glass chamber constructed to fit a Forma Scientific model 2095 water bath. This chamber was filled with

11 liters of either the water from the fishes' acclimation tank or water from the tap. Tap water was left in the test chamber overnight under constant aeration prior to introducing fish. Fish were introduced to the chamber at their acclimation temperature. The unit was then set to either heat or cool. The heating rate is approximated by the line, $Y = 1/5X + 23.5$, where the variable X represents time in minutes and Y represents temperature in Celcius. The overall heating rate of 0.2 C per minute approximates the heating rate of Cyprinodon milleri in a CTMax determination of that fish (Otto and Gerking, 1973). Lowe and Heath (1969) used a somewhat more rapid heating rate of 0.5 C per minute in CTMax tests of Cyprinodon macularius. The cooling rate used in my testing was about the same as the heating rate, 0.2 C per minute. The cooling rate is approximated by the line, $Y = -1/5X + 23.5$. CTMin was not determined for either C. milleri or C. macularius.

During testing, aeration was continuous to eliminate temperature stratification and oxygen depletion. Fish were observed regularly and frequent measurements of temperature and dissolved oxygen levels were made. When the fish lost equilibrium or were no longer coordinated in their swimming activity, they were immediately removed to their acclimation temperature. The point at which balance or swimming became impaired was taken as the CTM for that fish. The CTM for each acclimation temperature was calculated as the mean of all the individual CTM's. Data were analyzed for significant differences between acclimation temperatures by means of a "t" test.

Oxygen Tolerance

The tolerance of the Pahrump killifish to low levels of dissolved oxygen was determined by observing the behavior of fish as oxygen was removed by sodium sulfite. The sulfite ion binds the oxygen according to the equation: $2\text{SO}_3 + \text{O}_2 \longrightarrow 2\text{SO}_4$. Calculations based upon gram molecular weights indicate that 7.89 ppm of sulfite are required to bind 1 ppm of dissolved oxygen. Lewis (1970) used this method on a number of fishes, observing little difference between the behavior of fish in chambers in which oxygen had been depleted by the standard method of bubbling the water with nitrogen versus oxygen depletion by sulfite. He concluded that the levels of sodium sulfite needed to deplete the water of oxygen did not constitute a significant osmotic challenge to the fish.

My tests were conducted in a rectangular, 5 liter aquarium half filled with water. The water in the test aquarium was within $\pm 1^\circ\text{C}$ of the water temperature at which the fish were acclimated. The available water surface for diffusion of oxygen was 240 square millimeters. Tests were begun within one hour of introduction of the fish into the test chamber. The total time for any one test was seldom over 90 minutes. In my experiments 500 milligrams of sodium sulfite were usually enough to completely remove the oxygen. Two or three fish were used in each test. Some tests were conducted with a 1.5 mm mesh wire screen placed approximately 3 cm under the water surface which prevented the fish from reaching the water-air interface where diffusion would supply the surface layer with oxygen. In other tests fish were allowed to rise to the surface. Oxygen

levels were monitored with a polarographic YSI model 57 oxygen analyzer and probe fitted with a stirrer which, along with swimming movements of the fish, served to keep the oxygen content of the water fairly uniform. Oxygen levels which caused fish to come to the surface were recorded, as were the oxygen levels which caused equilibrium loss and spasmodic swimming movements. Fish were removed before death and were returned to their aquarium after a 24 hour observation period in a separate container. The fish were weighed to the nearest gram on a triple beam balance.

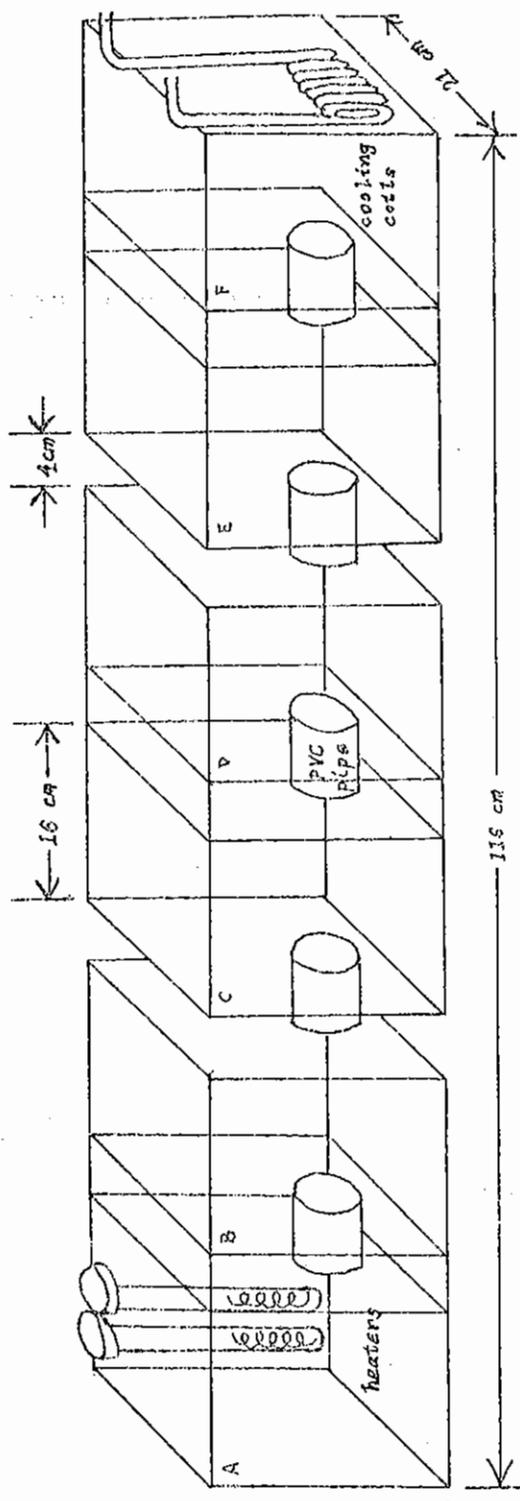
Temperature Preference

Methods of measurement of the preferred temperature of fishes are diverse, but most let fish voluntarily choose the water temperature it prefers. Apparatus for these experiments include shallow, baffled, horizontal troughs, vertical columns, and complex systems which respond to movements of fish by either raising or lowering the water temperature.

My temperature preference apparatus was constructed from three 20 liter aquaria (figure 1). Each aquarium was divided into two chambers using glass partitions with circular holes near the bottom large enough to admit a section of 2 inch I.D. PVC plastic pipe. The pipe, 4 cm in length, was sealed into place using silicone rubber aquarium sealant. The three partitioned aquaria were connected to one another lengthwise in a similar manner. The aquarium bottoms were covered with a layer of gravel banked up to create a gradual incline to the passages between chambers. One of the end chambers was equipped with a copper coil through which chilled water was circulated. The chamber at the opposite end of the apparatus was fitted with two 100 watt, thermostatically controlled, aquarium heaters. The effect of heating at one end and cooling at the opposite end set up a temperature gradient of 20 to 25 C range. Each chamber was supplied with an air stone to assure mixing and limit vertical stratification.

Fish were placed into the apparatus in the evening in the chamber closest to their acclimation temperature and their distribution recorded after approximately one hour. The following day, 12 to 13 hours later, observations were made at about one hour intervals.

Figure 1. Details of construction of temperature preferendum apparatus used in this study.



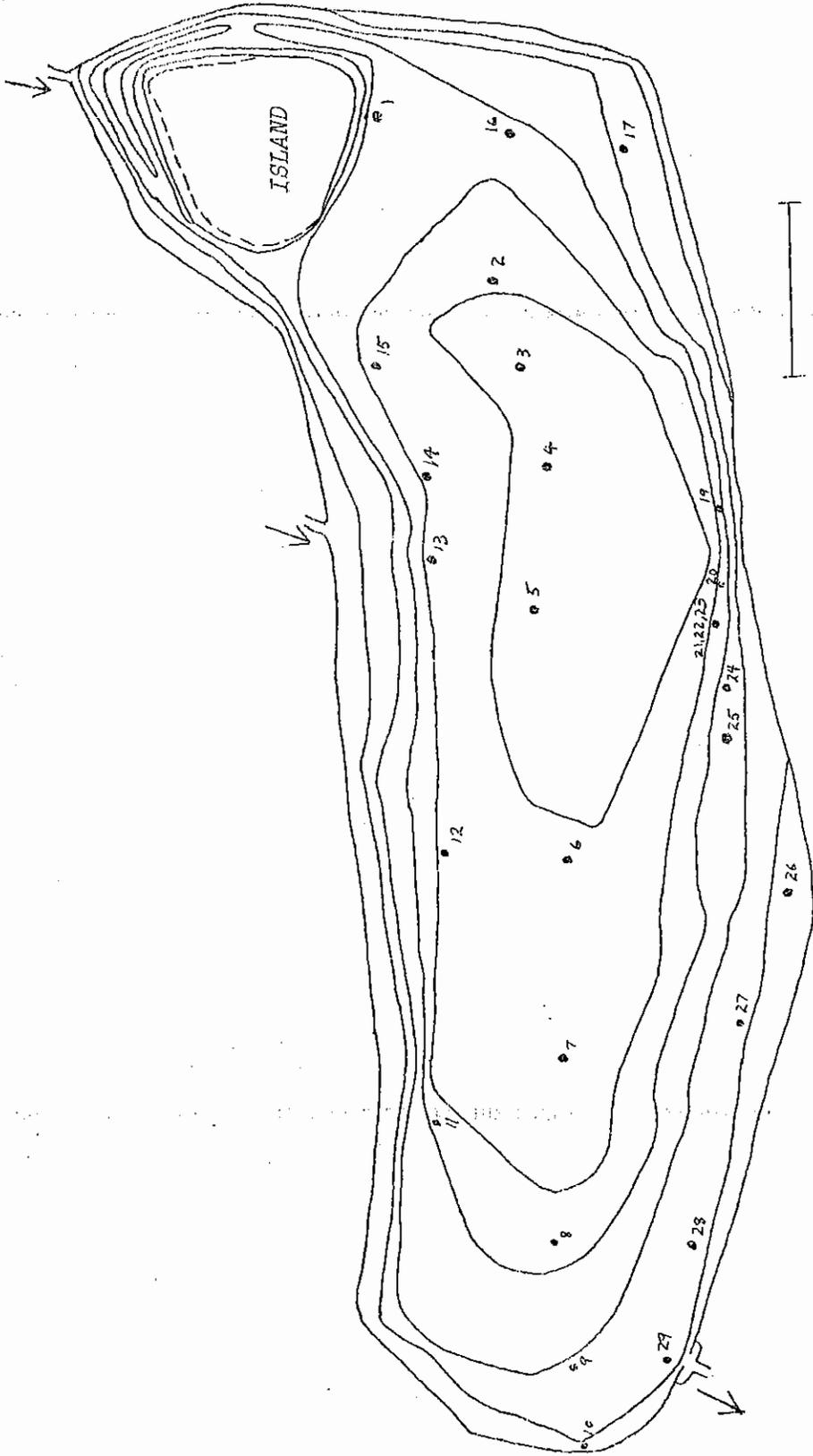
Temperatures were measured with a mercury thermometer and recorded. The following day, observations were continued and the fish were removed at the end of the day. The possibility of loss of acclimation and the problem of feeding the fish was avoided by limiting the testing to two days.

Distribution at Corn Creek Pond

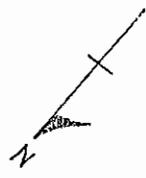
Trapping was conducted in November 1976 and in January 1977 to document the effect of temperature and dissolved oxygen on the distribution of Pahrump killifish in Corn Creek Pond. In November, 32 Trophy, 42 cm minnow traps were placed throughout the pond at various depths (figure 2). Each trap was lined with 1.5 mm mesh wire screen and baited with either liver or fish-flavored canned cat food. Three of these traps were placed in each of the three springheads. Traps were checked daily for the presence of fish. Before disturbing the water by moving the trap, dissolved oxygen and temperature measurements were taken with a YSI model 57 oxygen analyzer. These measurements were made as close to the trap as possible. Trapping in November was concluded after three days. In January, when water temperatures had cooled somewhat, trapping lasted only two days. Procedures followed in January were identical with November procedures except that traps 20, 21, 22, 23, 27, and those traps in the springheads were eliminated. Data were analyzed by least squares analysis of variance and determination of the correlation coefficient.

Figure 2. Map of Corn Creek Pond located on the Desert National Wildlife Range showing trap locations and pond contours.

Corn Creek Pond
Desert Wildlife
Range
Contours at 1 foot



0 30
scale in feet



U.S. GEOLOGICAL SURVEY

DESCRIPTION OF CORN CREEK POND

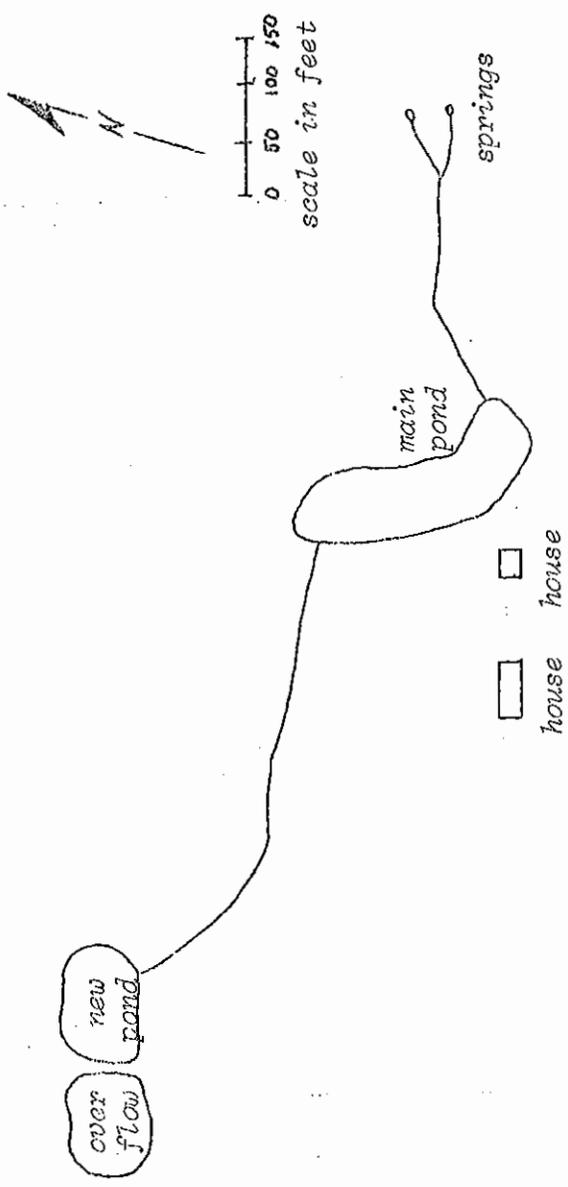
Corn Creek Pond is located at the Desert National Wildlife Range field headquarters, approximately 30 miles northwest of Las Vegas, Nevada on U. S. Highway 95. This man-made impoundment was constructed over 40 years ago to serve presumably as a reservoir for farming operations. The pond was mapped using a plane table survey method as outlined in Welch (1948) to obtain the physical dimensions of the pond and to provide a way of locating traps (figure 2). The surface area of the pond is approximately 0.18 hectares with a total volume of roughly 1600 cubic meters. The bottom tapers gradually to a maximum depth of nearly 2 meters. During the study the pond also contained an island in its southeastern most portion. This has since been connected to the shore by the filling of a channel between the island and the bank. The bottom of the pond consists of a layer of silt rich in organic matter and up to 50 cm deep. This produces anoxic conditions and hydrogen sulfide is generated. Deacon and Minckley (1974) state that production of hydrogen sulfide is relatively common in desert aquatic habitats.

The pond has, as a water source, three springs of constant temperature (21 C) and dissolved oxygen content (4.5 ppm). Volume of flow from the springs is about 125 gpm with the majority of the water supply for the springs derived from the Sheep Mountain Range to the east (Fiero, 1975). The two springs providing the greatest amount of flow to the pond are located approximately 105 meters east of the pond. A third smaller spring, approximately 50 meters east of the pond and north of the other two springheads, provides a negligible

volume of water. Prior to construction of new channels, the three springs joined to follow a single course into the pond at a point near the middle of the eastern bank. In January, 1977, concrete-lined channels were installed in an effort to reduce the loss of flow due to infiltration into the soil and to evapotranspiration by a dense stand of the common reed, Phragmites communis, lining the original course. The springhead with the least flow has been put in a separate channel which flows into the pond near the original inflow. The other two spring flows now converge 17 meters from their sources and flow into the pond at the southeast corner near the former island. The outflow runs through a weir located at the northwest end of the pond. This outflow provides water for another pond which has recently been constructed (figure 3).

The main pond has shown seasonal temperature variations from 26 C in July, August, and September 1976, to a low of 14.8 C in January 1977. Vertical temperature stratification in the pond was limited to 3-4 C when measured in November 1976. Because of its shallow depth, the pond probably does not become much more stratified in the summer. Dissolved oxygen levels at Corn Creek in summer and fall are highly variable due to dense submerged vegetation. The pond water was frequently supersaturated during the study. In November, oxygen levels were recorded from less than 0.5 ppm at the pond bottom to more than 17 ppm near the surface. Saturation level for oxygen at this time was near 8.4 - 8.9 ppm. In January, oxygen levels had become less variable, with levels recorded from 6.8 to 15 ppm. Saturation in January was from 8.7 to 9.4 ppm, depending upon the temperature.

Figure 3. Location of springs and ponds at the Desert National Wildlife Range field headquarters at Corn Creek.



50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000

Similar observations of supersaturation have been recorded in shallow ponds containing macrophytes (Hannon and Anderson, 1971).

Vegetation in the pond consists of pondweed, Potamogeton sp., spiny naiad, Najas marina, and Chara sp., with Chara being the dominant form during the months the pond was observed. Also present in quantity throughout the pond is the alga, Spirogyra sp., as well as lesser amounts of Oscillatoria sp. Emergent vegetation consists predominately of Phragmites communis which is densest along the east and south shores. A small stand of cattails, Typha sp., occurs near the point of the new main inflow channel. Water cress, Nasturtium sp. is common in the vicinity of the pond outflow. The pond is heavily shaded by large willows, Salix sp., two of which extend their branches over a third of the width of the pond and in autumn contribute many leaves to the water. The roots of these trees grow into the water and provide cover for the killifish.

Invertebrates are common in the pond. Dragonfly and damselfly naiads predominate. The common pond snail, Physa virgata, is present in large numbers. In the two main springheads exists another snail of the family Hydrobiidae. It is apparently an undescribed form endemic to the Corn Creek Springs (J. J. Landye, unpublished data, 1975).

Vertebrates present include the bullfrog, Rana catesbeiana, and its tadpoles which are abundant. Prior to March 1976, a single painted turtle, Chrysemys picta, was present in the pond. A large population of mosquitofish, Gambusia affinis, were eliminated in March and April 1976. The Pacific treefrog, Hyla regilla is also known to occur at the pond, but it is listed as rare.

A checklist of birds of the Desert National Game Range lists a large number of aquatic birds. Fifteen of these birds are known to have fish eating habits. Of these fifteen, the eared grebe, the pied-billed grebe, the snowy egret, the belted kingfisher, and the common merganser could, perhaps, have the greatest effect on the killifish at Corn Creek. These birds are not known to be year round residents of the pond and, therefore, their influence would be limited to migratory stopovers (G. T. Austin, personal communication, 1977).

RESULTS

Critical Thermal Limits

Data obtained in measurements of the CTMax and the CTMin of the Pahrump killifish were analyzed by a "t" test. All acclimation temperatures showed significant differences from one another on the basis of these values. In the analysis of CTMax, the 16 C acclimation temperature was omitted due to an extreme variance in the data. In analyzing CTMin, acclimation temperatures of 13.5 and 16 C were combined as were the 21 and 23.5 C data and the 24.5 and 25 C data. CTM data is summarized in table 1. Since CTMax and CTMin were not always measured at the same acclimation temperature, some values are missing. CTMin values for 13.5 and 16 C acclimation temperatures are represented as being less than 1.5 C since at that temperature the fish still maintained their equilibrium. The experimental set up did not furnish temperatures below 1.5 C. Ranges represent the temperature range between the CTMax and CTMin value for an acclimation temperature. Range was calculated when both CTMax and CTMin were known.

Table 1. Summary of CTMax and CTMin data for the Pahrump killifish.

| Acclimation temperature (C) | CTMax (C) | CTMin (C) | Range (C) |
|-----------------------------|-----------|-----------|-------------|
| 11.5 | 30.0 | --- | --- |
| 13.5 | --- | >1.5 | --- |
| 16.0 | 30.9 | >1.5 | 30.0 |
| 21.0 | 34.2 | 4.3 | 29.9 |
| 23.5 | 37.6 | 4.2 | 33.4 |
| 24.5 | --- | 5.8 | --- |
| 25.0 | 38.4 | 5.9 | 32.5 |
| 31.0 | 40.0 | 6.9 | <u>33.1</u> |

$\bar{X} = 31.8$
 $SD = 1.7$

11-11-81 10:00 AM

Oxygen Tolerance

The results of oxygen tolerance tests on the Pahrump killifish indicate their ability to withstand low levels of dissolved oxygen down to 1.0 ppm. Oxygen tolerance results are presented in table 2. An inadequate supply of oxygen for the fishes respiratory needs was indicated when the fish came to the surface for the richer oxygen source provided by diffusion at the air-water interface. This behavior was termed "surfacing." When a wire screen was present, preventing the fish from using the oxygen rich surface layer, the fish became frantic bumping the screen with their heads. The point at which these fish attempted to reach the surface was defined as surfacing behavior. These fish more frequently lost their equilibrium during the testing than did fish which had the surface available to them. Fish with access to the surface could sustain themselves for a number of minutes at the surface while subsurface oxygen levels were at or near 0 ppm. Measurements of respiratory rates by observing opercular activity indicate a constant rate during testing even as oxygen fell below 1.0 ppm. Similar constant respiratory rates were reported by Feldmeth and Baskin (1975) during oxygen tolerance tests of the unarmored stickleback.

Table 2. Results of oxygen tolerance experiments on the Pahrump killifish, Empetrichthys latos latos.¹

| Weight of fish (g) | "Surfacing" O ₂ ppm | Loss of equilibrium | Screen | Temperature (C) |
|--------------------|--------------------------------|---------------------|--------|-----------------|
| 0.85 | 1.0 | 0.2 | yes | 23.0 |
| 0.90 | 1.2 | no ² | no | 23.2 |
| 0.90 | 1.2 | no ² | no | 23.2 |
| 1.20 | 1.0 | 0.2 | yes | 23.0 |
| ----- | 1.0 | no ² | no | 25.5 |
| ----- | 1.0 | no ² | no | 25.5 |
| 3.25 | 1.0 | 0.2 | yes | 22.5 |
| 4.10 | 1.0 | 0.2 | yes | 22.5 |
| 4.80 | 0.8 | 0.2 | yes | 22.5 |
| 5.30 | <u>0.9</u> | 0.2 | no | 23.2 |
| | $\bar{X} = 1.0$ | | | |

1 Missing weights were between 1 and 3 grams.

2 Loss of equilibrium not recorded because fish were removed to prevent death.

Temperature Preference

The results of attempting to define a preferred temperature for the Pahrump killifish are presented in figures 4, 5, and 6. These figures represent fish acclimated to approximately 14, 25, and 30 C respectively. Data are presented as the combination of a three degree temperature range resulting in seven groups. The 3 degree ranges in the diagrams were arbitrarily chosen for convenience. The groups are not intended to represent the individual chambers of the apparatus, but rather the distribution of fish throughout the entire apparatus. This was done in order to include the fish that were observed in the connecting pipe between individual chambers. The data for day one and day two of the tests are separated in order to better observe any day to day difference in fish distribution. Prior to commencement of testing, fish placed in the apparatus with all chambers at the fish's acclimation temperature showed no special preference for one chamber or another and swam freely between all chambers. When testing was begun, some preference was shown for chamber F where the cooling coil was located. The coil provided cover for the fish while in other chambers no such hiding place was available. Other than this observation, the fish did not seem to prefer any one chamber over another regardless of the temperature.

Figure 4. Frequency distribution of Pahrump killifish in temperature preferendum apparatus when acclimated to approximately 14 C.

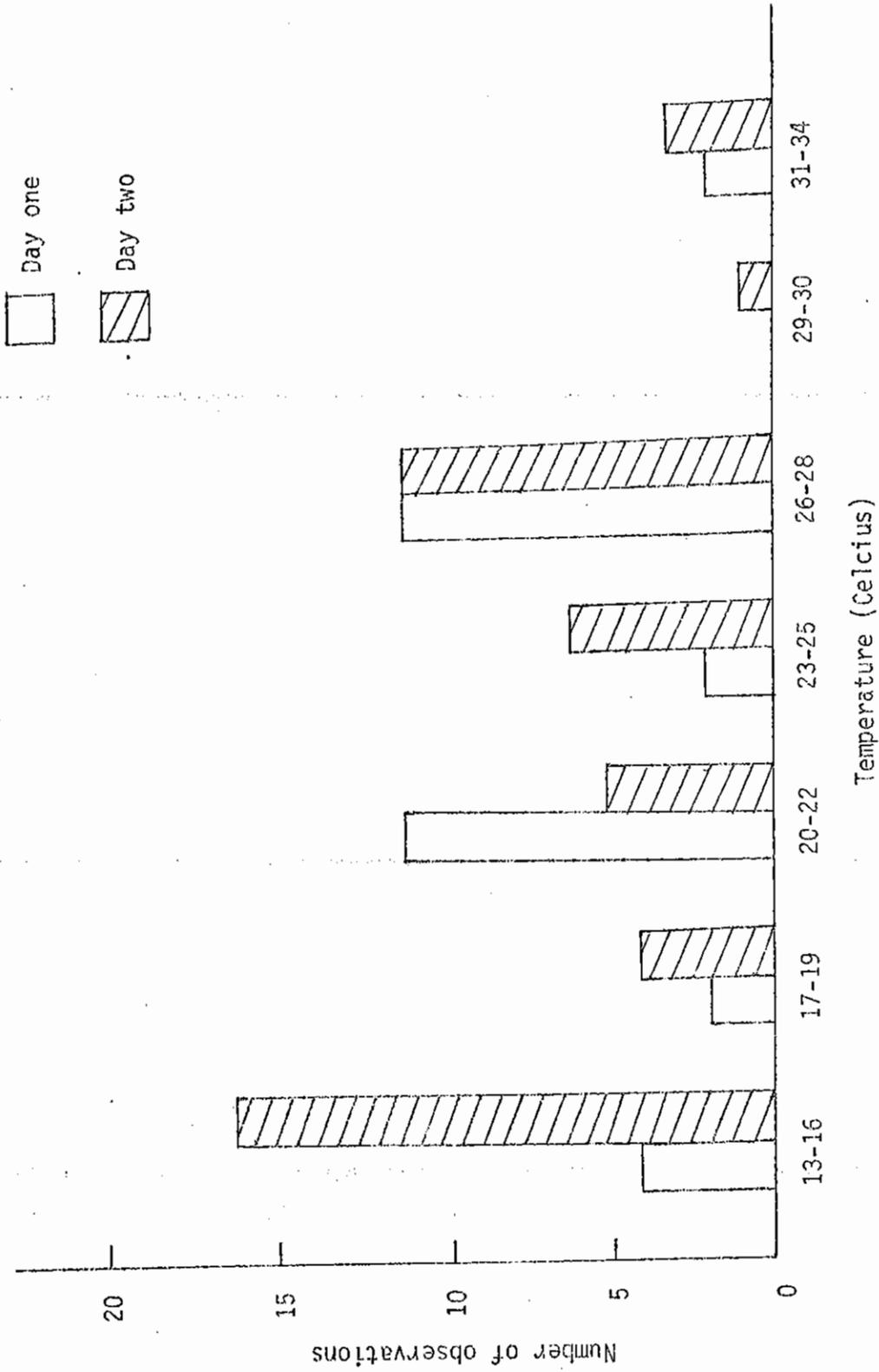


Figure 5. Frequency distribution of Pahrump killifish in temperature preferendum apparatus when acclimated to approximately 25 C.

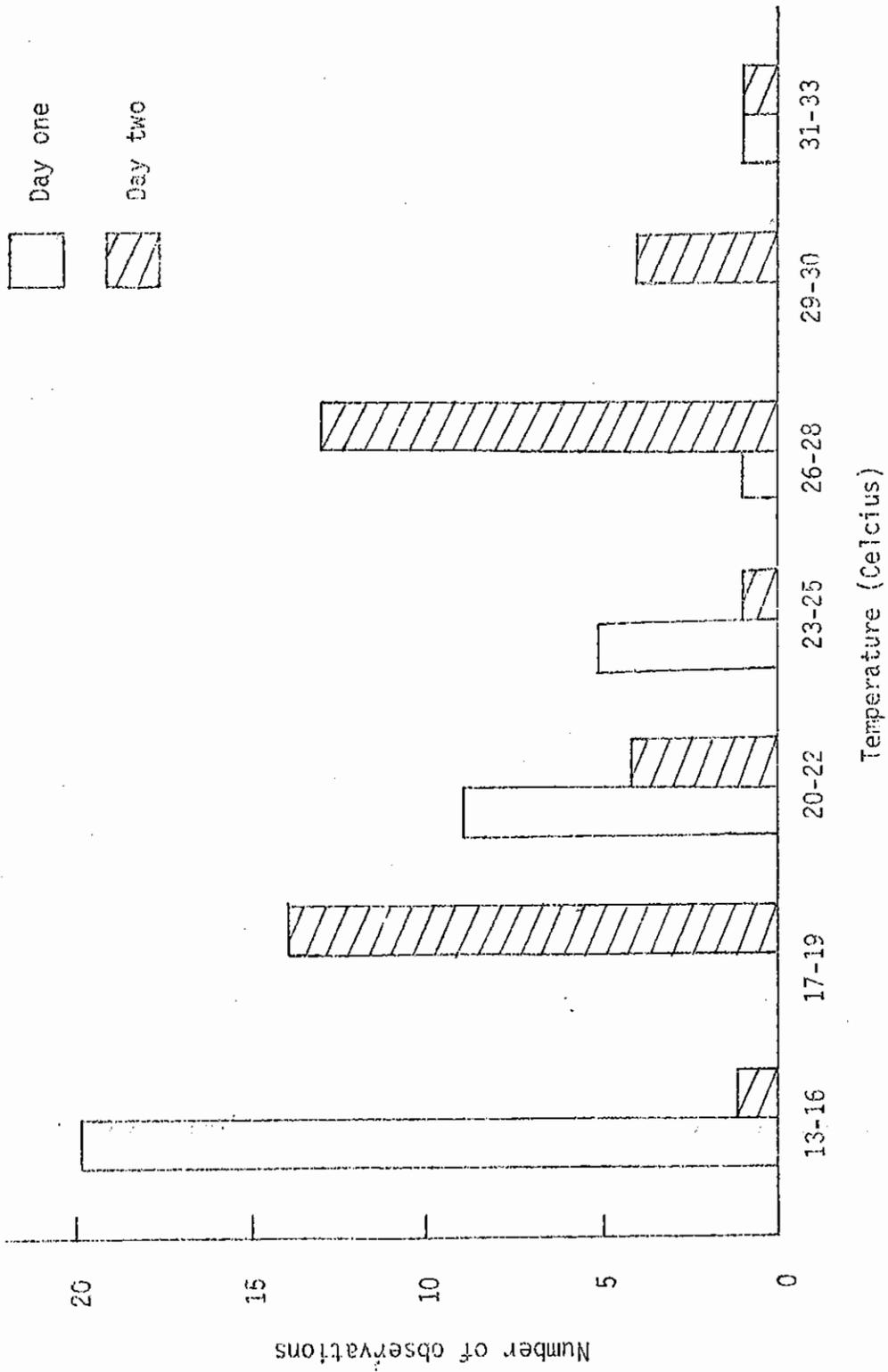
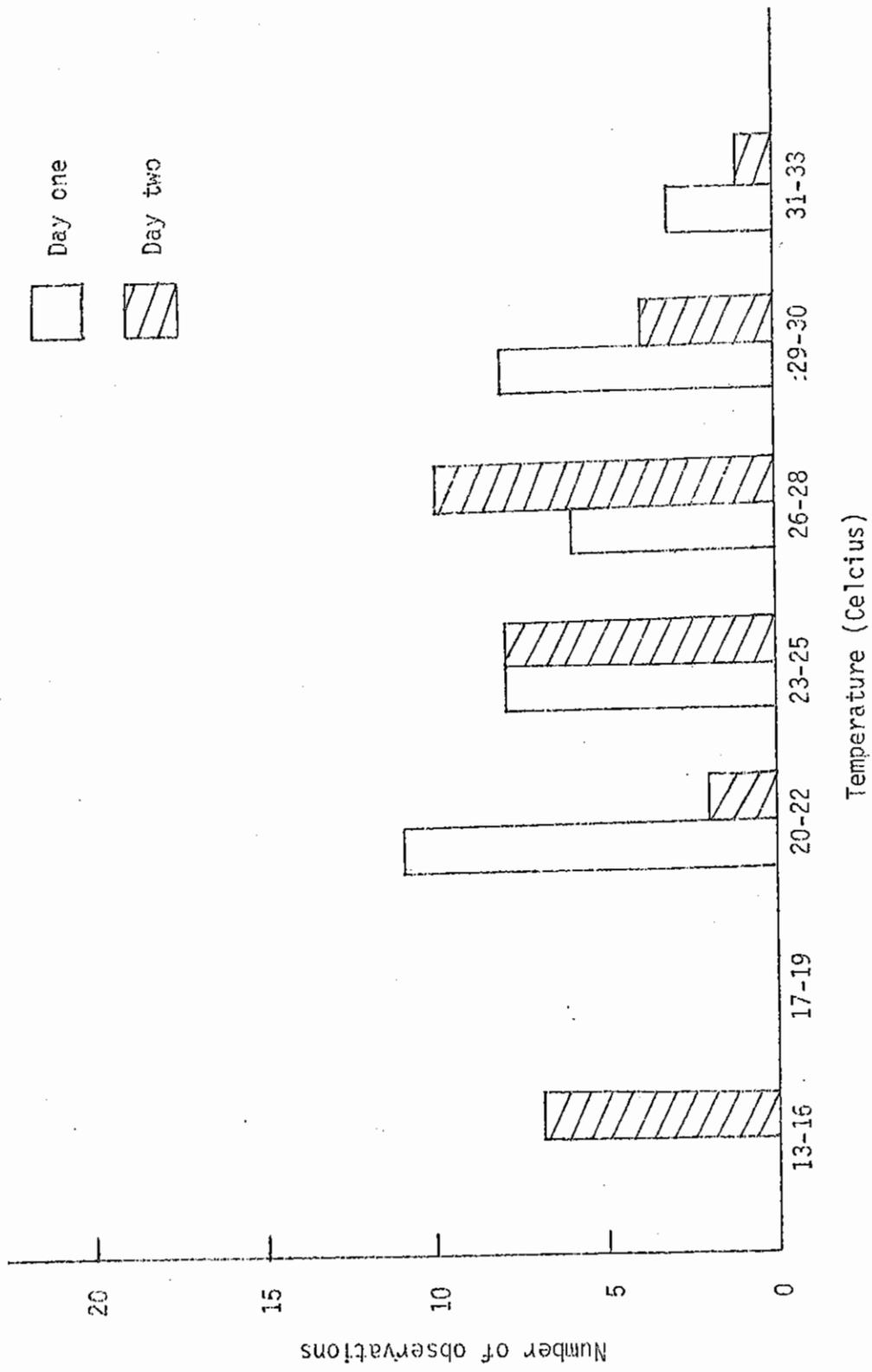


Figure 6. Frequency distribution of Pahrump killifish in temperature preferendum apparatus when acclimated to approximately 30 C.



01-04
05-08
09-12
13-16
17-19
20-22
23-25
26-28
29-30
31-33

Distribution at Corn Creek Pond

The results of trapping during November 1976 and January 1977 are presented in figures 7 and 8. These frequency histograms indicate an apparent higher rate of capture at certain water temperatures. In November, when the average pond temperature was about 17.7 C, traps with water temperature between 17 and 19 C showed the highest number of fish per trap hour, while in January data show a higher catch per hour at temperatures between 15 and 17 C. The average water temperature in January was about 14.8 C. This apparent correlation between water temperature and fish catch is not significant when analyzed statistically. While there is a statistical difference between temperatures at individual traps at the 0.05 probability level, there is no difference in rate of capture at the different temperatures.

The results of my attempt to document the effects of oxygen on the distribution of killifish in Corn Creek Pond are presented in figures 9 and 10. In November, an extreme range in oxygen levels existed at the pond with some traps in water practically devoid of oxygen and some in water supersaturated with oxygen. The data presented in the frequency histograms of figures 9 and 10 indicate an apparent influence of oxygen upon catch per trap hour. November catches were highest in waters above 9 ppm during daylight hours and in January, when no traps were in water below 6 ppm dissolved oxygen, the greatest catch per trap hour occurred again above 9 ppm. The lack of traps in water with oxygen below 6 ppm is due to traps not being forced to the bottom of the pond in some locations as they

Figure 7. Distribution of killifish in Corn Creek Pond related to temperature for November 1976.

Figure 8. Distribution of killifish in Corn Creek Pond related to temperature for January 1977.

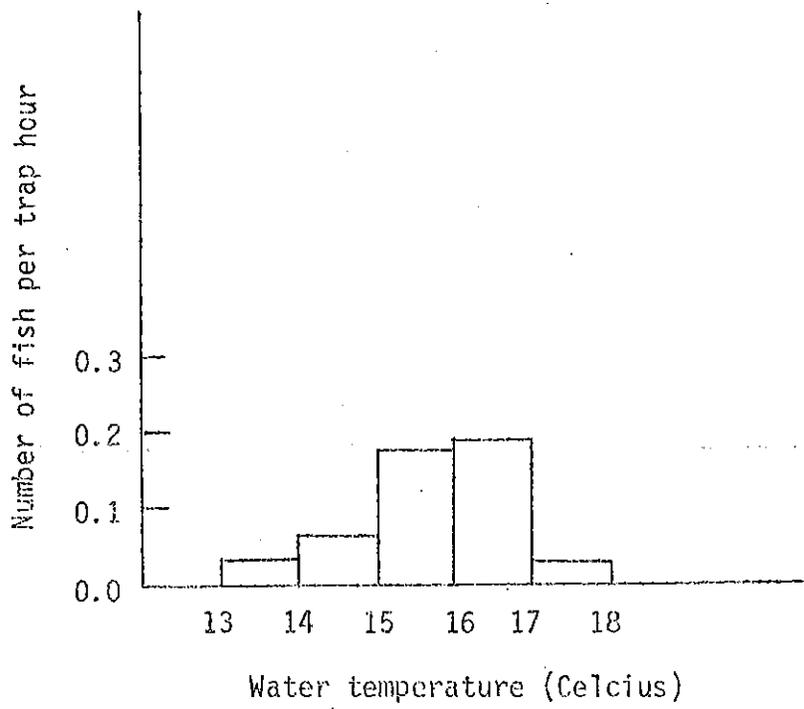
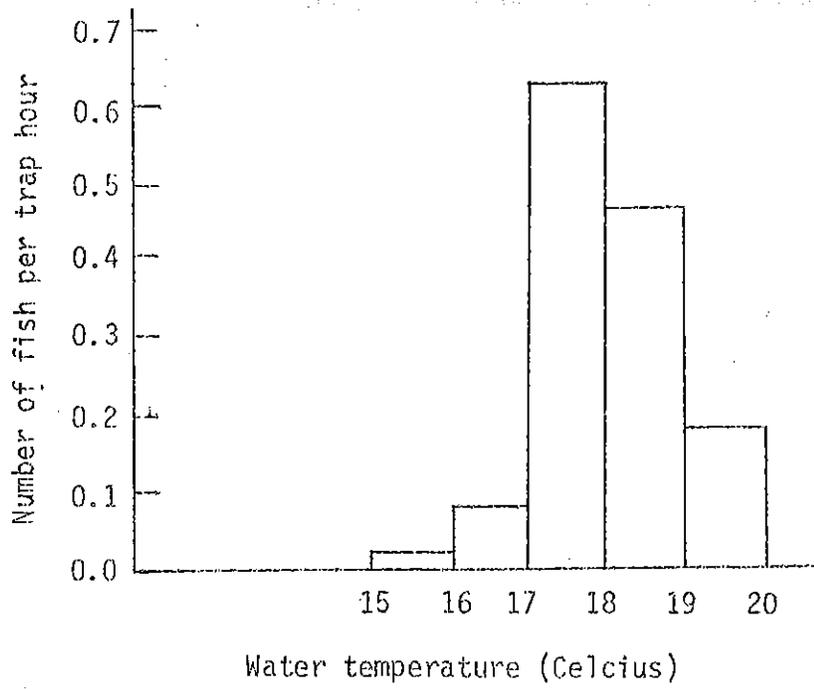
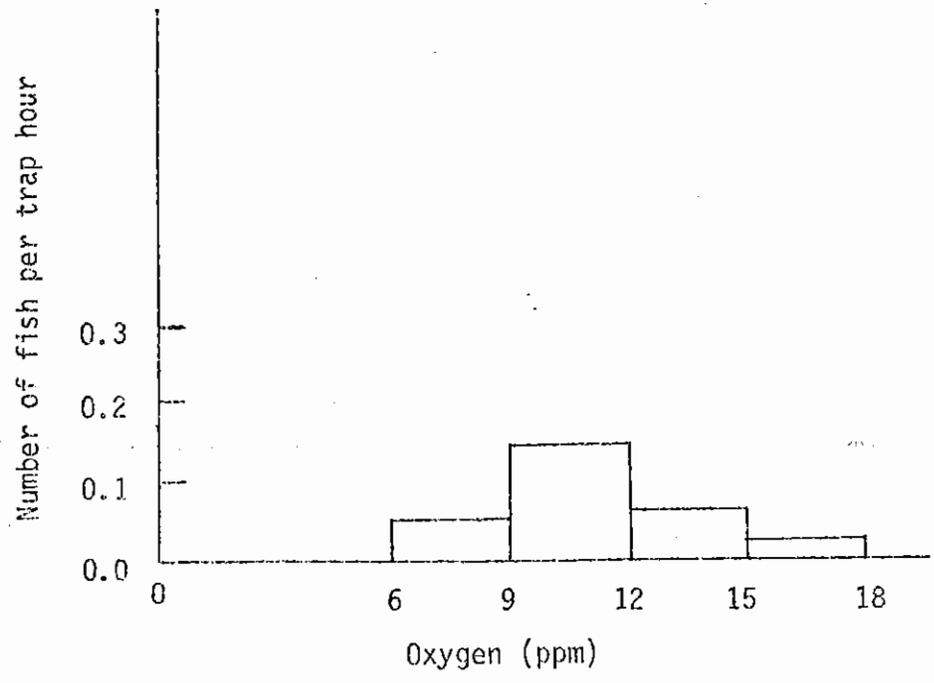
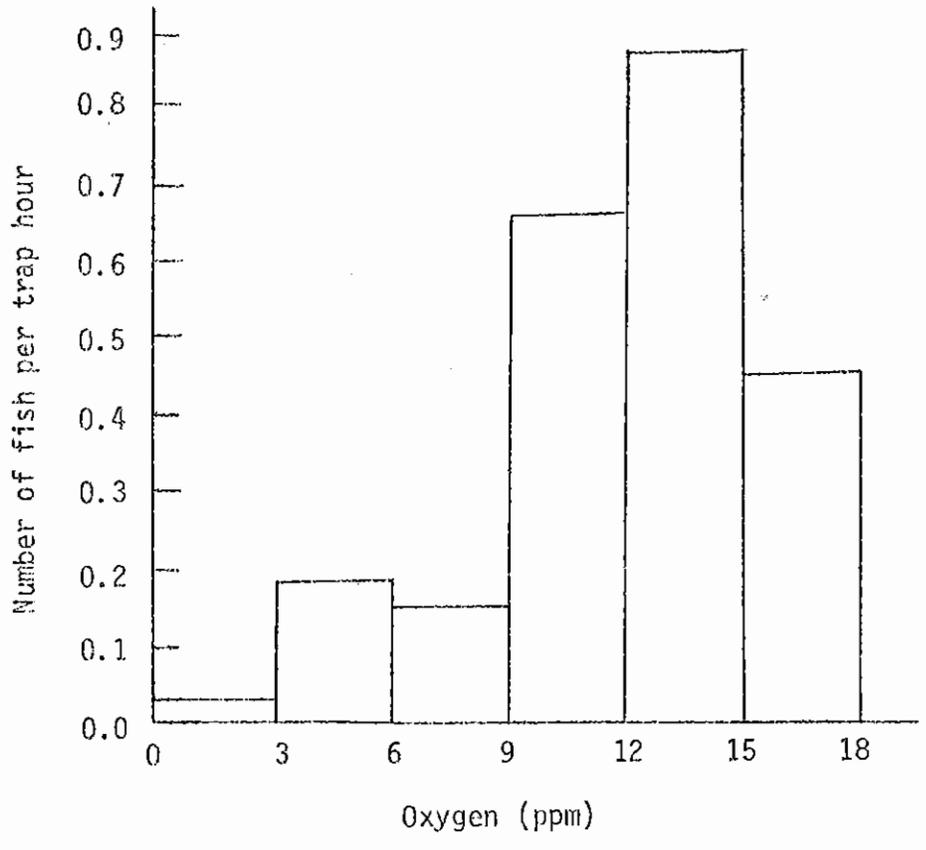


Figure 9. Distribution of Pahrump killifish in relation to dissolved oxygen levels in Corn Creek Pond in November 1976.

Figure 10. Distribution of Pahrump killifish in relation to dissolved oxygen levels in Corn Creek Pond in January 1977.



had been in November. A correlation analysis of both sets of data indicates that only in November 1976 did oxygen levels influence distribution of fish at Corn Creek Pond. The r value for the month of November is 0.424, with confidence limits of 0.226 and 0.588 at a probability level of 0.01. The r^2 value of 0.18 indicates that approximately 18 percent of the time oxygen levels were responsible for the observed distribution of fish in the pond.

DISCUSSION

Critical Thermal Limits

In their various habitats, fishes are exposed to a wide range of temperatures, from those of the Antarctic where some fish show no distress when supercooled to -2.5 C (Somero and DeVries, 1967), to fishes of the southwestern deserts, where Cyprinodon atrorus has been observed in water of 47.2 C (Deacon and Minckley, 1974). The native habitat of the Pahrump killifish at Manse Spring was a constant, warm 23.5 C prior to its failure. It has been speculated that springs in this area have had constant temperatures for thousands of years (Brown and Feldmeth, 1970). If so, the Pahrump killifish has a wider range of thermal tolerance than might be anticipated for an animal under such constant conditions. This wide range of temperature tolerance was clearly demonstrated in tests of CTMax and CTMin conducted upon the fish.

In tests of CTMax, the Pahrump killifish showed a mean increase in CTMax of 0.6 C for each 1 C increase in acclimation temperature over the range of 11.5 to 31 C . Fry, Brett, and Clawson (1942) reported a 0.3 C increase in upper lethal temperatures of goldfish, Carassius auratus, with every 1 C increase in acclimation temperature. In the speckled trout, Salvelinus fontinalis, only a 0.14 C increase in upper lethal temperature was noted for an increase of 1 C in acclimation temperature. For Cyprinodon milleri, an increase in acclimation temperature from 15 to 35 C produced a mean increase in CTMax of 0.4 C (Otto and Gerking, 1973). The catfish, Ictalurus punctatus, demonstrated a similar mean increase of 0.4 C per degree

increase in acclimation temperature over the range of 21 to 32 C (Cheetham et al., 1976). It is evident that the rate of gain of thermal tolerance as related to acclimation level of the Pahrump killifish is similar to rates calculated for C. auratus, C. milleri, and I. punctatus. Only the speckled trout has a distinctly lower rate and this is most likely attributable to the much lower thermal limits of salmonids in general (Brett, 1956).

As mentioned earlier, the ultimate lethal temperature is the point at which any further increase in acclimation temperature fails to affect the upper temperature tolerance. Figure 11 demonstrates an apparently related phenomenon where increasing the acclimation temperature above 25 C has little effect upon the CTMax. CTMax data plotted for C. milleri (figure 12) show a similar leveling off trend as acclimation temperatures exceed 15 C. This implies that at or near a 25 C acclimation temperature the ultimate CTMax is reached for the Pahrump killifish, while C. milleri is approaching its ultimate CTMax at acclimation temperatures near 15 C. The ecological significance of this might have to do with the habitat of C. milleri where drastic daily temperature fluctuation occur on a regular basis (Naiman, Gerking, and Ratcliff, 1973). In some areas water temperatures may fluctuate as much as 14.8 C between day and night. If C. milleri is to survive, it must retain a wide thermal tolerance. Perhaps equally important is the ability to tolerate almost its upper critical limit when acclimated to only 15 C. At this temperature the fish would also probably retain the ability to survive drastic temperature minima that might occur at night. The wide tolerance

Figure 11. Critical thermal maximum temperatures of E. latos at various acclimation temperatures.

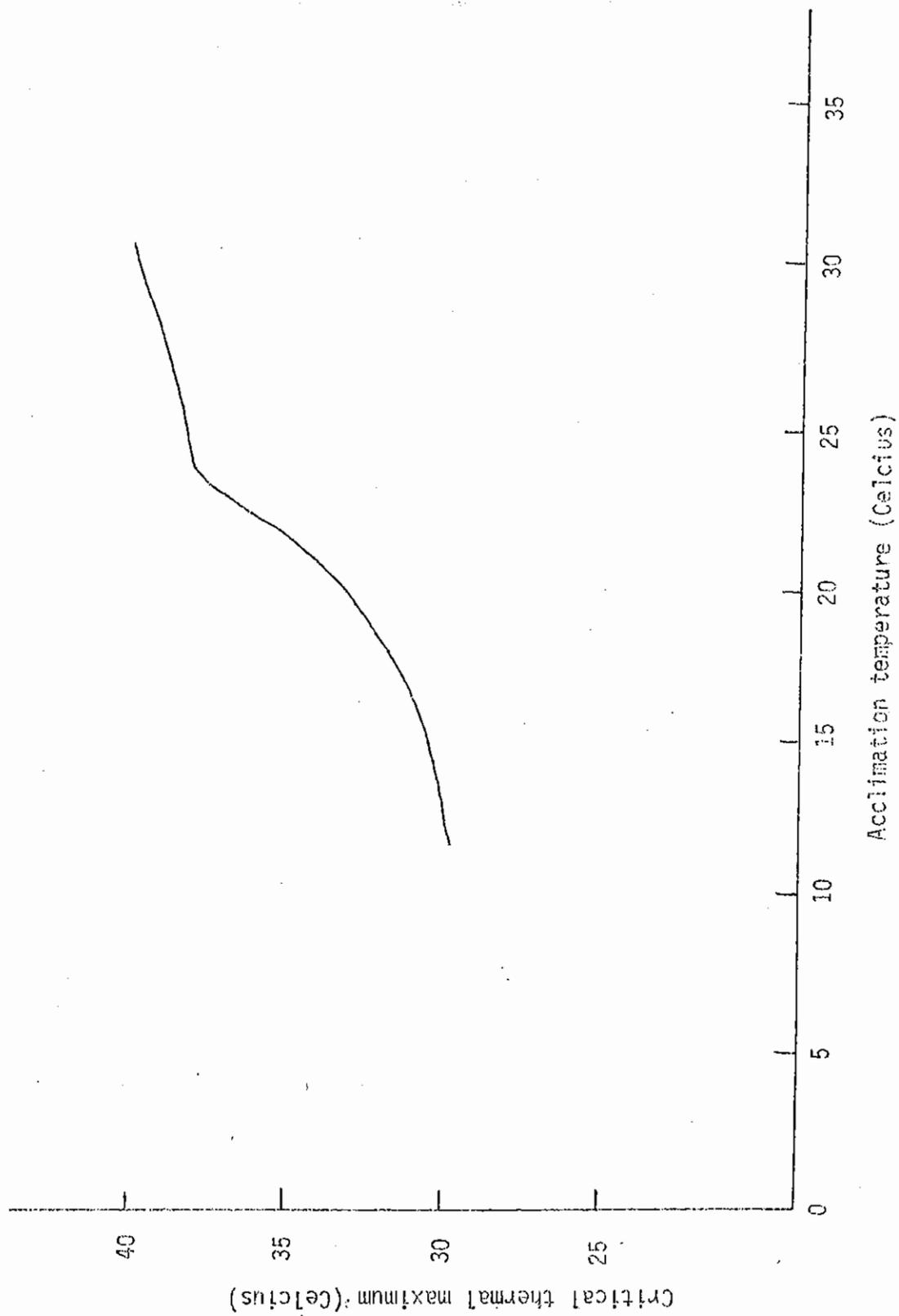
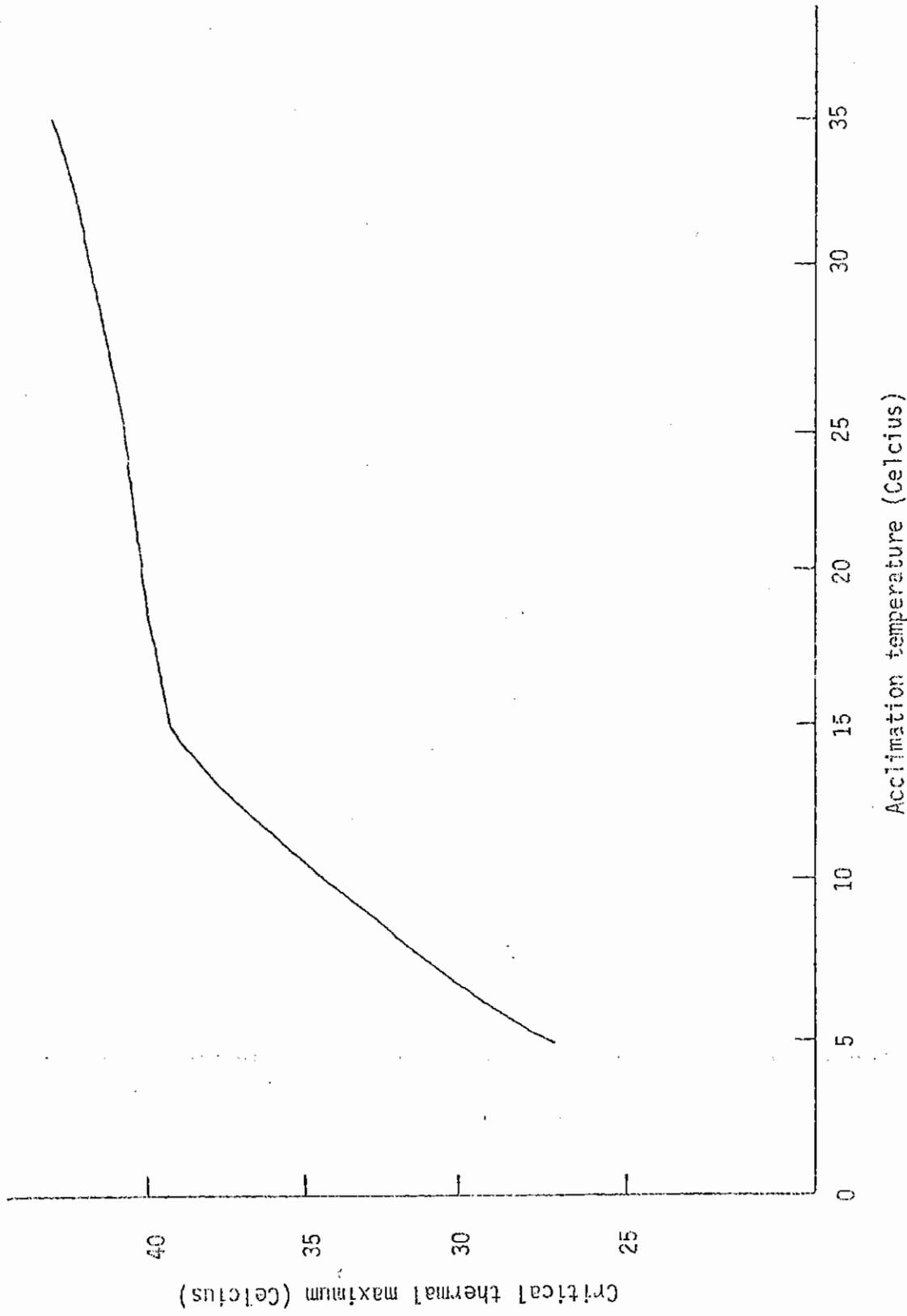


Figure 12. Critical thermal maximum temperatures of Cyprinodon milleri at various acclimation levels (from Otto and Gerking, 1973).



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would also expand the habitat of the fish by decreasing temperature barriers to movement.

The Pahrump killifish, however, occurred in a much less severe environment where temperature fluctuations were minimal. This species does not gain its full heat tolerance until an acclimation temperature of 25 C is reached. At that point much of the species' cold tolerance has been lost.

Comparison of the CTMax of the Pahrump killifish with other species for which CTMax has been determined (table 3) places it below Cyprinodon in thermal tolerances compared at acclimations of about 30 C. CTMax of the killifish is about the same as that of I. punctatus at 30 C, and of Rhinichthys oculus and Blennius pholis at acclimation temperatures of 20 to 21 C. The CTMax of the killifish exceeds that of Gasterosteus aculeatus at acclimations of 22.7 to 23.5 C.

Records of CTMin were found only for B. pholis (Campbell and Davies, 1975). The difficulties in measurement of lower lethal limits have been mentioned by Fry, Hart, and Walker (1946). The problem is the physical property of freshwater which causes it to freeze at 0 C, often before the lower limits of cold tolerance of the fish have been reached. Campbell and Davies used a marine species in salt water to obtain CTMin values as low as -1.3 C. The Pahrump killifish was able to tolerate temperatures as low as 1.5 C without a loss of equilibrium when acclimated to 16.0 C and lower. Fish at these temperatures were almost inactive and the determination that CTMin had not been reached was made by upsetting the fish with

Table 3. Critical thermal limits for some selected fishes.

| Species | Acclimation temperature | CTMax | CTMin | Authors |
|-------------------------------|-------------------------|---------|-------|---------------------|
| <u>Fundulus heteroclitus</u> | 10.5-15 | 40.5-42 | --- | Huntsman and Sparks |
| <u>Cyprinodon macularius</u> | 30 | 42.0 | --- | Lowe and Heath |
| | 32 | 42.5 | --- | |
| | 34 | 43.2 | --- | |
| <u>Cyprinodon milleri</u> | 5 | 27.2 | --- | Otto and Gerking |
| | 15 | 39.2 | --- | |
| | 25 | 40.7 | --- | |
| | 35 | 43.1 | --- | |
| <u>Gasterosteus aculeatus</u> | 8.0 | 30.5 | --- | Feldmeth and Baskin |
| | 13.0 | 32.5 | --- | |
| | 15.6 | 33.7 | --- | |
| | 18.6 | 33.5 | --- | |
| | 22.7 | 34.6 | --- | |
| <u>Blennius pholis</u> | 10.0 | 32.5 | -1.3 | Campbell and Davies |
| | 20.0 | 35.1 | 4.9 | |
| <u>Rhinichthys osculus</u> | 20.5 | 35.0 | --- | Starkey et al. |
| <u>Ictalurus punctatus</u> | 12.0 | 34.5 | --- | Cheetam et al. |
| | 20.0 | 35.5 | --- | |
| | 24.0 | 37.5 | --- | |
| | 28.0 | 39.5 | --- | |
| | 32.0 | 41.0 | --- | |

a glass rod. In all cases, the fish was able to right itself. As acclimation temperatures were increased, an increase in CTMin was observed (table 1). This increase was somewhat slower than in the case of the CTMax. The rate of increase in CTMin per degree increase in acclimation temperature averaged only 0.4 C. Leveling off of the CTMin at low acclimation temperatures can be seen in figure 13, where acclimation temperatures below 16 C produced no apparent changes in CTMin. This may be interpreted as an approach to the ultimate CTMin. If this is true, then the Pahrump killifish is able to withstand temperatures approaching freezing for short periods when acclimated to 16 C. Temperatures below 4 C are unlikely to be encountered by the Pahrump killifish under natural conditions because habitats occupied by them are not known to form an ice cover in winter.

With data for both CTMax and CTMin of the Pahrump killifish, a critical thermal range (CTR) can be calculated (table 1). This range has a mean value of 31.8 ± 1.7 C. This represents the average range between CTMax and the CTMin of the fish at the tested acclimation temperatures. Blennius pholis has a calculated CTR of 32 ± 2.5 C. Although both fish show a similar CTR, not enough data on CTMin of fishes exist to be able to make generalizations about these values.

In figure 14, I have taken liberties with a method of quantifying the zone of thermal tolerance of fishes devised by Brett (1956). In place of lethal temperatures I have used CTM values which for CTMax normally seem to show higher values than lethal temperatures on the order of 5 C. In construction of the diagram, acclimation temperatures

Figure 13. Critical thermal minimum temperatures of E. latos at various acclimation temperatures.

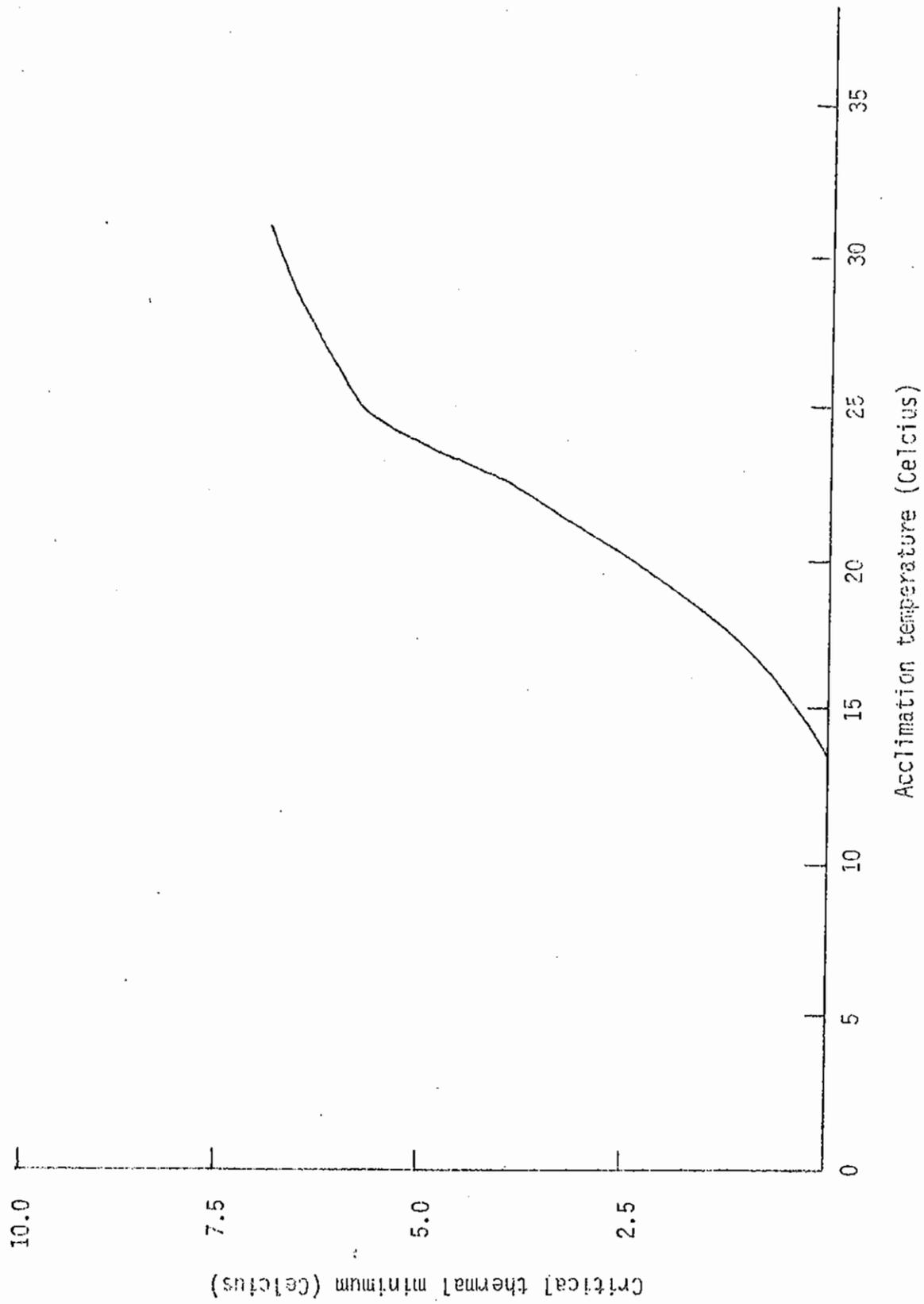
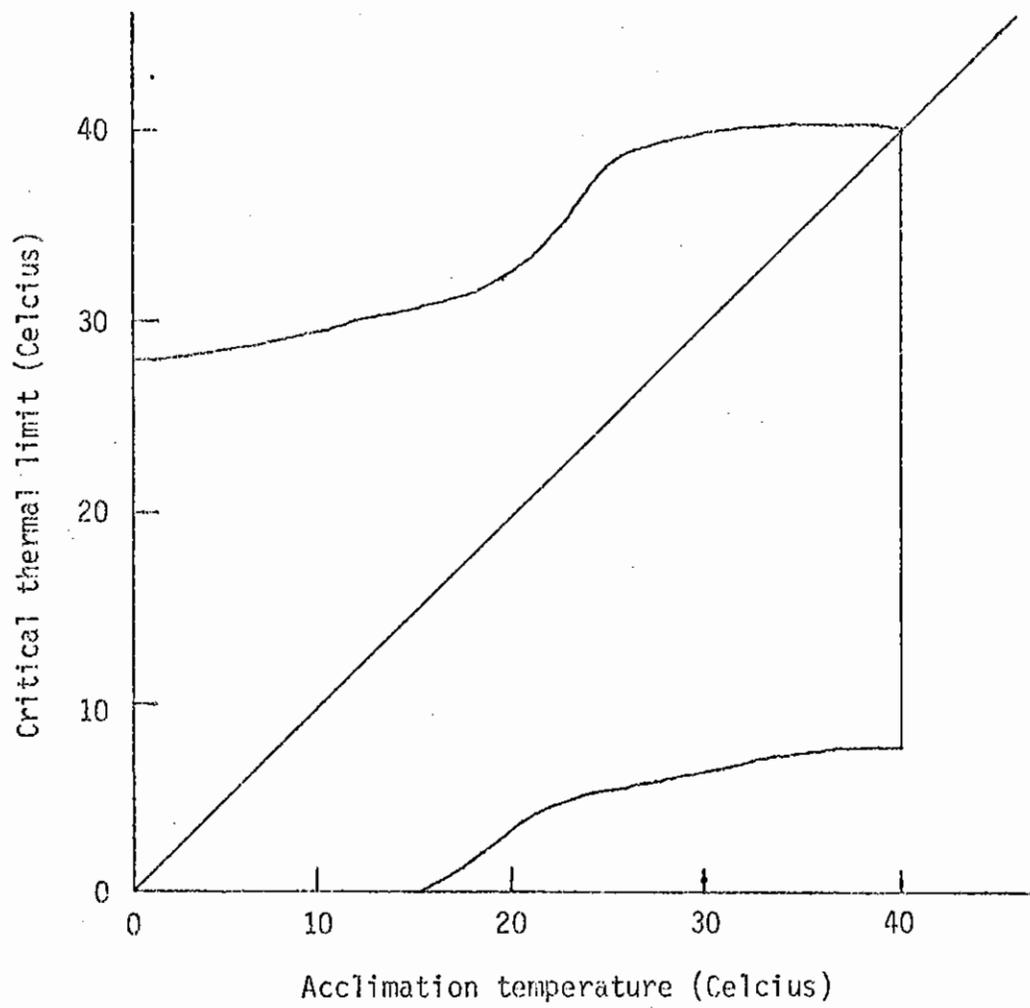


Figure 14. Zone of critical temperature for the Pahrump killifish,
Empetrichthys latos latos.



of 16 C and below are represented as having an estimated CTMin value of 0 C. Above acclimation temperatures of 31 C, the CTMin is extrapolated to a CTMin of approximately 8 C. For CTMax, a low of approximately 28 C at acclimation of 0 C is assumed. The CTMax curve is extrapolated at the high end to meet a 45 degree line from the origin. From this point, where the CTMax meets the 45 degree line, a vertical line is dropped to intercept the CTMin curve. The area enclosed is the critical thermal zone representing roughly 1218 degrees squared for the Pahrump killifish. It may be impossible to acclimate killifish to temperatures of 38 C as presumed in the diagram, however, the construction of the zone of tolerance is based upon the temperature curves intercepting the 45 degree line from the origin. Compared to the same measurements of this area based upon lethal temperatures calculated by Brett (1956) for other species, the Pahrump killifish fares rather well. Salmonids have a zone of about 500 C², cyprinids of from 750 for Notropis atherinoides to 1220 for the goldfish, Carassius auratus. No values for cyprinodontids are presented in Brett's data.

Oxygen tolerance

Lewis (1970) has studied the adaptations of fishes to conditions of oxygen depletion, observing similar use of the surface layer by fishes as demonstrated in my tests with the Pahrump killifish. He defined four categories of adaptations to survive oxygen depletion. Category one fish have no adaptations to survive oxygen depletion and are unsuccessful at using the surface layer. Micropterus salmoides is a category one fish. Carassius auratus represents a category two fish, physiologically adapted to tolerate oxygen depletion, but still unable to utilize the surface layer. Category three includes Notemigonus crysoleucas, Brachydanio rerio, Lepomis macrochirus and Lepomis cyanellus, all fishes which are partially anatomically adapted to survive oxygen depletion by being of a body form that allows limited use of the surface layer. Category three fish must expend much energy to maintain the proper orientation to the surface and because of this cannot survive extended periods of oxygen depletion. Category four includes Fundulus notatus, Gambusia affinis, and Poecilia reticulata, all forms suited to survival for extended periods in water devoid of oxygen. By virtue of their dorsally oriented mouths they are very efficient at using the surface layer and are under little stress in oxygen depleted water. The Pahrump killifish, although a close relative of Fundulus, has a body shape and mouth orientation which makes utilization of the surface layer difficult. The Pahrump killifish best fits category three as a partially anatomically adapted species.

The survival value of a high tolerance of low oxygen levels in Manse Spring is unknown since available records indicate oxygen levels

were seldom below 7 ppm. It may be that this tolerance is retained from earlier periods when Manse Spring may have had different characteristics than it has in recent years. (This possibility is explored in a later portion of this discussion.)

Crenichthys, however, is a frequent inhabitant of springs with high temperatures and low oxygen. This fish seems to have a similar oxygen tolerance to that of the Pahrump killifish. If Crenichthys is forced to remain in water of 0.7 ppm at 32 C death results (Hubbs, Baird, and Gerald, 1967). A determination of lethal oxygen tolerances was not possible with the Pahrump killifish. Although they would have been of interest, restrictions on the sacrifice of this fish did not permit this sort of experimentation. However, measurements that were made indicate that oxygen must exceed 1.0 ppm in habitats occupied by Pahrump killifish. Corn Creek Pond as described in an earlier section was characterized by highly variable oxygen levels in November 1976 and January 1977 and possibly at other times of the year as well. Portions of Corn Creek Pond do not meet the oxygen requirements of the Pahrump killifish as evidenced by deaths of killifish during trapping operations at the pond.

Although nocturnal oxygen levels were not recorded at Corn Creek it is safe to assume that areas of high photosynthetic activity during the day will have high levels of respiration at night. The results of such activity would be the creation of zones of low oxygen in these areas at night. This would necessitate the movement of killifish out of these areas to avoid the possibility of suffocating. During trapping in November, traps 3, 13, 20, 21, and 22 all contained dead

killifish on one or two of the three days of trapping. These deaths must have been due to oxygen depletion during the night since there was no sign of physical damage to the fish and the occurrence of six dead fish in a single trap precluded the possibility of chance fatality. The oxygen levels at these five traps averaged only 2.4 ppm during the day over the three days of trapping. Thus, while the fish may have been attracted to the baited trap during the day when oxygen levels permitted occupation of these areas, at night when the vegetation began to consume oxygen they were unable to escape and suffocated. Laboratory results indicate that the oxygen level must have been at or below 1.0 ppm during the night for these deaths to have occurred. Also observed in the lab was the movement of fish to the surface where water richer in oxygen existed, as test chamber oxygen fell below 1.0 ppm illustrating what Fry (1947) called a "directive factor", that is, a factor which directs an organism out of an area of possibly lethal conditions. This same factor must be operating in Corn Creek Pond during the night when fish respond to lowered oxygen levels by movements into areas of higher oxygen. The data obtained in November illustrated the influence of oxygen upon the distribution of killifish.

At UNLV the typical bimodal activity pattern of the Pahrump killifish reported by Deacon and Minckley (1974) is absent during the winter when the outdoor pools fall to temperatures of as low as 6 C. Oxygen may be acting as a directive factor in these pools as it probably is in Corn Creek. The fish at these lowered temperatures typically remain within thickets of aquatic macrophytes in the pools during the day, however, at night the fish can be seen swimming in open water. It is

possible here too that a gradient of oxygen from the thickets to the clear water is causing the movement of fish and resulting in an apparent nocturnal activity pattern. As temperatures exceed approximately 20 C the fish again become active during the day.

Temperature preference

The ability of fish to recognize and select temperatures they prefer has long been recognized (Bull, 1936; Ferguson, 1954). Experiments since the late 1940's have centered around the determination of preferred temperatures and the "temperature preferendum". The temperature preferendum is the point at which the acclimation temperature is the same as the preferred temperature (Fry, 1947). These values have been determined for a wide variety of fishes in a wide variety of apparatus. By attaching thermocouples to the dorsal spine of the brown bullhead, Ictalurus nebulosus, a final preferendum to between 29 and 31 C was determined in a laboratory gradient (Crawshaw, 1974). McCauley and Tait (1970) used a vertical gradient to determine the final preferendum of lake trout, Salvelinus namaycush, to be 11.7 C. In a horizontal gradient, Javid and Anderson (1966) determined a final preferendum of 17 C for Atlantic salmon, Salmo salar.

Using my apparatus results were obtained that indicate the Pahrump killifish lacks behavioral thermoregulatory abilities. It seems that the killifish lacks the sensory ability that would enable it to detect and select appropriate water temperatures. This conclusion is based upon three lines of evidence. First, to enter an adjacent chamber a fish would have to cross a steep gradient of up to 4.5 C in a distance of 4 cm. Fish were observed to cross this barrier freely as if it did not exist. Second, short term experimentation with another fish, Relictus solitarius, under the same conditions in this apparatus show this fish to also move between chambers, but to prefer certain chambers at temperatures somewhat lower than their acclimation

level. The temperatures preferred by R. solitarius acclimated to approximately 24 C fell between 18 and 22.5 C. When R. solitarius was chased into a chamber of higher temperature its immediate response was to turn around and reenter its original chamber or to actively swim about apparently seeking refuge from the increased temperature. Pahrump killifish exhibited no such behavior when chased into increased temperatures. Increased respiratory movements could be noted but the fish did not seek cooler temperatures and often remained in the chamber for hours. Third, on two occasions killifish moved into water above their lethal temperature and remained there until dead. On one occasion a single fish acclimated to 30 C was found in chamber A at 36 C, a temperature within the critical thermal limits of the fish, but exceeding its lethal limits. The second occurrence involved two fish acclimated to approximately 14 C which were found dead in chamber A at 36.5 C. CTMax data indicate chamber A was about 6 degrees above the CTMax for these fish. Upon entering the 36.5 C chamber the fish must have become disorientated almost immediately and died shortly thereafter. In both cases the movement must have taken place during the night or early morning since the fish were found upon arrival to the lab around 0800. Since the chamber temperature was relatively constant, these deaths involved the fish voluntarily swimming into lethal temperatures rather than becoming trapped in a chamber of increasing temperature.

In addition to laboratory results the field data obtained in November and January trapping at Corn Creek Pond point to a lack of behavioral thermoregulation. No significant differences in fish catch as related to temperature were found to exist. The field results in

themselves are not sufficient to conclude that the fish in Corn Creek do not select particular water temperatures due to the limited period of the study, but in conjunction with lab results the conclusion seems valid.

Cherry, Dickson, and Cairns (1975) were unsuccessful at determining thermal responsiveness in two species of Catostomidae, Catostomus commersoni and Hypentelium nigricans, and two species of Percidae, Etheostoma blenniodes and Percina crassa. They did, however, determine preferred temperatures for 13 other species, indicating that apparatus and procedures were adequate. Thus, the Pahrump killifish does not stand alone in its lack of behavioral thermoregulatory capabilities. Other members of the Cyprinodontidae are suspected to actively thermoregulate. Cyprinodon milleri may move into areas of its habitat shaded by salt crusts where water temperatures are cooler than unshaded areas (Deacon and Minckley, 1974). Barlow (1958) studied C. macularius in the Salton Sea and related fish movements and distribution to water temperature. And Naiman (1976) reported selection of certain temperatures by the Amargosa pupfish, Cyprinodon nevadensis amargosae, in a thermal stream.

This study is the first of thermoregulatory behavior for a cyprinodontid fish from a relatively constant temperature environment. Further testing of species isolated for long periods in environments of stable temperature may show similar results.

The tolerance retained by the Pahrump killifish in its fairly constant and uniform native environment leads to speculation as to why this tolerance was not lost. If, as Brown and Feldmeth (1970) supposed,

the spring temperatures have been constant for thousands of years one might conclude, as they did, that the evolutionary retention of tolerances to temperature presents no handicap to the fish despite its uselessness. It does not seem to require any extra expenditure of energy on the part of the fish during development.

A second possibility although difficult if not impossible to document, is that the springs in this area have not always been thermal. Perhaps, over hundreds or thousands of years water temperatures have fluctuated as geologic activity altered the hydrogeology of the area. In such a case the wide tolerance of temperature demonstrated by Pahrump killifish would be necessary for survival.

A third possibility is that of variable flow rates of the springs (J.E. Deacon, personal communication, 1976). Under such conditions high flow periods could result in the creation of shallow marsh type habitats over large areas. In addition, significant changes in flow would be expected to result in variability in temperatures of the springhead. The fish inhabiting marsh habitats would encounter daily and seasonal fluctuations of oxygen as well as temperature. Fish inhabiting springheads would encounter long term changes in temperature, but oxygen fluctuations would be encountered daily.

The apparent loss of the thermoregulatory ability, as evidenced by temperature preference tests, may reflect adaptation to an environment that does not present a thermal challenge. This loss of behavioral temperature regulation ability would suggest fairly constant temperature conditions in the native habitat for extended periods of time. In such a habitat the energy expended in the development of a temperature

detection system could be conserved. On the other hand the retention of a wide zone of thermal tolerance suggests that long term thermal variation may have been a significant environmental challenge to which the species has responded.

The loss of the ability to effectively thermoregulate via behavior may present a burden to Pahrump killifish in transplant sites of non-uniform temperatures. If lethal temperatures exist in an unstable habitat a fish kill may occur as killifish swim into water above their thermal limits, become disoriented and die, similar to results obtained in laboratory tests. In all of the present transplant sites the lack of ability to respond to temperature has not caused any hardship to the killifish. Present habitats either fluctuate within a range of temperatures within the tolerance of the Pahrump killifish or they fluctuate widely but are of such a morphometry that they are fairly uniform in temperature throughout, making the ability to select temperatures useless. What has made the Pahrump killifish successful in such habitats is its wide thermal tolerance range which allows it to acclimate to seasonal temperature regimes.

SUMMARY

1. The Pahrump killifish has the ability to tolerate temperature ranges from 0 C to 40 C for short periods of time when properly acclimated.
2. The Pahrump killifish is apparently incapable of behavioral thermoregulation.
3. The Pahrump killifish can tolerate levels of oxygen down to 1.0 ppm without adverse affects and can be classified as a category three fish according to Lewis's scheme.
4. Oxygen had a significant influence upon fish distribution in the Corn Creek Pond in November 1976 when oxygen levels influenced fish distribution approximately 18 percent of the time.

LITERATURE CITED

- Barlow, G.W. 1958. Daily movements of desert pupfish, Cyprinodon macularius, in shore pools of the Salton Sea, California. Ecology 39, 580-587.
- Brett, J.R. 1944. Some lethal temperature relations of Algonquin Park fishes. Univ. Toronto Stud., Biol. Ser. 52, 1-49.
- Brett, J.R. 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol. 31, 75-87.
- Brown, J.H. and C.R. Feldmeth 1970. Evolution in constant and fluctuating environments: Thermal tolerances of desert pupfish, Cyprinodon. Evolution 25, 390-398.
- Bull, H.G. 1936. Studies on conditioned responses in fishes VII. Temperature perception in Teleosts. J. Mar. Biol. Ass. U.K. 21, 1-27.
- Campbell, C. and P.S. Davies 1975. Thermal acclimation in the teleost, Blennius pholis (L). Comp. Biochem. Physiol. a Comp. Physiol. 52, 147-152.
- Cheatham, J.L., C.T. Garten Jr., G.L. King, and M.H. Smith 1976. Temperature tolerance and preference of immature channel catfish, Ictalurus punctatus. Copeia pp. 609-612.
- Cherry, D.S., K.L. Dickson, and J. Cairns Jr. 1975. Temperatures selected and avoided by fish at various acclimation temperatures. J. Fish. Res. Bd. Can. 32, 485-492.
- Crawshaw, L.I. 1975. Attainment of the final thermal preferendum in brown bullheads acclimated to different temperatures. Comp. Biochem. Physiol. a Comp. Physiol. 52, 171-174.
- Deacon, J.E. and S. Bunnell 1970. Man and pupfish. Cry Calif. 5, 14-21.
- Deacon, J.E. and W.L. Minckley 1974. Desert Fishes. In "Desert Biology" (G.W. Brown Jr., ed.), Vol. II pp. 385-488. Academic Press, New York.
- Deacon, J.E., C. Hubbs, and B.J. Zahuranec 1964. Some effects of introduced fishes on the native fish fauna of Southern Nevada. Copeia pp. 384-388.
- Feldmeth, C.R. and J.N. Baskin 1976. Thermal and respiratory studies with reference to temperature and oxygen tolerance for the unarmored stickleback, Gasterosteus aculeatus williamsoni Hubbs. S. Calif. Acad. Sci. 75, 127-130.

- Ferguson, R.G. 1958. The preferred temperature of fish and their midsummer distribution in temperate lakes and streams. *J. Fish. Res. Bd. Can.* 15, 607-627.
- Fiero, W. 1975. Hydrogeology of Corn Creek Springs, Clark County, Nevada. Report to the U.S. Fish and Wildlife Service.
- Fry, F.E.J. 1947. Effects of the environment on animal activity. *Univ. Toronto Stud., Biol. Ser.* 55, 1-62.
- Fry, F.E.J., J.S. Hart, and K.F. Walker 1946. Lethal temperature relations for a sample of young speckled trout, Salvelinus fontinalis. *Univ. Toronto Stud., Biol. Ser.* 54, 1-35.
- Fry, F.E.J., J.R. Brett, and G.H. Clawson 1942. Lethal limits of temperature for young goldfish. *Rev. Canad. Biol.* 1, 50-56.
- Hannon, H.H. and B.T. Anderson 1971. Predicting the diel oxygen minimum in ponds containing macrophytes. *Progve. Fish. Cult.* 33, 45-47.
- Hart, J.S. 1947. Lethal temperature relations of certain fish of the Toronto region. *Trans. Roy. Soc. Can.* 41, 57-71.
- Hochachka, P.W. and G.N. Somero 1973. "Strategies of Biochemical Adaptation." W.B. Saunders Co.
- Houston, A.H. and D. Cyr 1974. Thermoacclimatory variation in the haemoglobin systems of goldfish, Carassius auratus, and rainbow trout, Salmo gairdneri. *J. Exp. Biol.* 61, 455-462.
- Hubbs, C. and J.E. Deacon 1964. Additional introductions of tropical fishes into Southern Nevada. *Southwest. Nat.* 9, 249-251.
- Hubbs, C., R.C. Baird, and J.W. Gerald 1967. Effects of dissolved oxygen concentration and light intensity on activity cycles of fishes inhabiting warm springs. *Amer. Midl. Natur.* 77, 104-115.
- Huntsman, A.G. and M.I. Sparks 1924. Limiting factors for marine animals 3. Relative resistance to high temperatures. *Contr. Can. Biol. N.S.* 2, 97-114.
- Javid, Y.M. and J.M. Anderson 1966. Thermal acclimation and temperature selection in Atlantic salmon, Salmo salar, and rainbow trout, Salmo gairdneri. *J. Fish. Res. Bd. Can.* 24, 1507-1513.
- Lewis, W.M., Jr. 1970. Morphological adaptations of cyprinodontids for inhabiting oxygen deficient waters. *Copeia* pp. 319-326.
- Lowe, C.H. and W.G. Heath 1969. Behavioral and physiological responses to temperature in the desert pupfish, Cyprinodon macularius. *Physiol. Zool.* 42, 53-59.

- McCauley, R.W. and J.S. Tait 1970. Preferred temperature of yearling lake trout, Salvelinus namaycush. J. Fish. Res. Bd. Can. 23, 1729-1733.
- Miller, R.R. 1948. The cyprinodont fishes of the Death Valley system of eastern California and southwestern Nevada. Misc. Publ. Mus. Zool. Univ. Mich. 68, 1-155.
- Miller, R.R. 1949. Desert fishes—Clues to vanished lakes and streams. Natur. Hist., N.Y. 58, 447-451 and 475-476.
- Miller, R.R. 1950. Speciation in fishes of the general Cyprinodon and Empetrichthys, inhabiting the Death Valley region. Evolution 4, 155-163.
- Minckley, W.L. and J.E. Deacon 1968. Southwestern fishes and the enigma of "endangered species". Science 159, 1424-1432.
- Naiman, R.J. 1976. Productivity of a herbivorous pupfish population (Cyprinodon nevadensis) in a warm desert stream. J. Fish Biol. 9, 125-137.
- Naiman, R.J., S.D. Gerking, and T.D. Ratcliff 1973. Thermal environment of a Death Valley pupfish. Copeia pp. 366-369.
- Otto, R.G. and S.D. Gerking 1973. Heat tolerance of a Death Valley pupfish. Physiol. Zool. 46, 43-49.
- Somero, G.N. and A.L. DeVries 1967. Temperature tolerance of some Antarctic fishes. Science 156, 257-258.
- Starkey, E.E., J.A. Kazmierski, M.R. Fletcher, and O.E. Maughan 1972. The effect of photoperiod on thermal resistance of speckled dace. Trans. Amer. Fish. Soc. 101, 715-718.
- Tsukuda, H. and W. Ohsawa 1974. Effects of temperature acclimation on the isoenzyme pattern of liver lactate dehydrogenase in the goldfish, Carassius auratus (L). Annot. Zool. Jpn. 47, 206-214.
- Uyeno, T. and R.R. Miller 1967. Relationships of Empetrichthys erdisi, a Pliocene cyprinodontid fish from California, with remarks on the Fundulinae and Cyprinodontinae. Copeia, pp. 520-532.
- Welch, P.S. 1948. Limnological Methods. McGraw-Hill Book Co., New York. 381 pp.