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ECHELLE, Anthony Allan, 1940-
BEHAVIOR AND ECOLOGY OF THE RED RIVER PUPFISH,
CYPRINODON RUBROFLUVIATILIS.

The University of Oklahoma, Ph.D., 1970
Zoology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

BEHAVIOR AND ECOLOGY OF THE RED RIVER PUPFISH, *CYPRINODON RUBROFLUVIATILIS*

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

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Norman, Oklahoma

1970

BEHAVIOR AND ECOLOGY OF THE RED RIVER PUPFISH,

CYPRINODON RUBROFLUVIATILIS

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ACKNOWLEDGEMENT

Sincere appreciation is extended to Dr. Loren G. Hill, my major advisor, for advice and unstinting encouragement throughout the course of this research. Special thanks are due Dr. Carl D. Riggs who provided timely encouragement and financial assistance during the period of my graduate work; he, along with Drs. Charles C. Carpenter, Leon S. Ciereszko, and Howard P. Clemens, read and criticized the manuscript. Financial support was provided by a graduate assistantship in zoology at the University of Oklahoma, two N.S.F. grant-in-aids at the University of Oklahoma Biological Station, a teaching position at Cameron State College, and a grant from the Oklahoma College Teacher Development Fund extended by the administration at Cameron State College.

Acknowledgement is also due Dr. Charles Taber and Messrs. Thomas Haislip, Phil Henderson, and Larry Renfro for various forms of assistance. Additional help was provided by Drs. George Barlow, James Deacon, and Douglas Martin.

My wife, Alice, cheerfully gave valuable assistance in helping me with field work, analysis of data, typing, and editing. Without her this dissertation would not have been completed.

TABLE OF CONTENTS

Chapter

LIST OF FIGURES

LIST OF TABLES

BEHAVIOR AND ECOLOGY OF THE RED RIVER PUPFISH, *CYPRINODON RUBROFLUVIATILIS*

CHAPTER I

INTRODUCTION

The Red River pupfish, *Cyprinodon rubrofluviatilis*, occurs in the upper reaches of the Red and Brazos rivers in Oklahoma and Texas (Moore, 1957). A similar form tentatively called *Cyprinodon pecosensis* by R. R. Miller (pers. comm.), is found in the Pecos River system of New Mexico and Texas.

Following Rosen's classification (1964), the Red River pupfish is a member of the family Cyprinodontidae superorder Atherinomorpha. *Cyprinodon*, with the monotypic genera *Jordanella* and *Floridichthys*, and several other genera, comprise the subfamily Cyprinodontinae (Myers, 1955).

The purpose of this paper is to describe the ecology and behavior of the Red River pupfish. Emphasis is placed on (1) the effects of temperature on the behavior of the population, (2) a description of behavioral motor patterns, (3) a description of territoriality, (4) a discussion of color patterns and associated behavior, and (5) inter-relationships between the pupfish and other fishes. Comparisons will be made with other members of the family, especially the Cyprinodontinae.

Liu (1965) published a short note on courtship behavior in *Cyprinodon*. He described behavioral differences between the species complex of the Death Valley system, typified by *C. macularius*, and the more eastern species complex of the United States, typified by *C. variegatus* and "*C. rubrofluviatilis*". His "*C. rubrofluviatilis*" were from a taxonomically nominal population in New Mexico that may not be conspecific with *C. rubrofluviatilis* of Oklahoma and Texas. The present description is based on observations of a single species, with emphasis on behavior in the field, while, as far as I have been able to determine, Liu's work dealt primarily with laboratory observations. Several authors (e.g., Kaufman and Kaufman, 1963; Kavanau, 1964) have noted that behavior in the field is frequently quite different from that observed under laboratory conditions; thus the value of field observations cannot be discounted. By concentrating on a single species, as I have done in this study, the investigator is likely to obtain a more detailed description of behavior in a particular species than might be gained from a more general study.

To my knowledge the only work dealing with the ecology of *C. rubrofluviatilis* is that of Martin (1968). He presented limited data on possible factors limiting its distribution as supporting information for a more comprehensive study of *C. variegatus*.

CHAPTER II

METHODS AND MATERIALS

This study is based primarily on observations of the fish in the natural environment. Data and conclusions based on laboratory observations are so noted. Observations began in March of 1967 with notes on localities of collection and general notes on behavior in the field and in aquaria. Field observations continued until November of 1969. During the period of this study, Red River pupfish were kept almost continuously in a variety of indoor and outdoor tanks from which extensive notes on behavior were taken. Color patterns were studied in greatest detail in the laboratory, but corroborating information was gained in the field.

In addition to field notebook and pen, field observations were recorded with the aid of (1) a portable "Unisonic" tape recorder, (2) a pair of 7 X 35 wide-angle binoculars, (3) a hand thermometer and a battery powered electrical thermometer, (4) a Lietz-Wetzlar 8-mm movie camera, and (5) a stenographer who recorded dictated observations as they were being made. "Stop-action" analysis of movies provided details of certain motor patterns (e.g., wrapping) that could not be discerned with the unaided eye.

Field observations of behavior were aimed primarily at observing the pupfish under all kinds of situations. The majority of the observations were of fish in the Prairie Dog Town Fork of the Red River near Childress, Childress Co., Texas and in Oscar Creek near Oscar, Jefferson Co., Oklahoma (referred to below as the "Childress" and "Oscar Creek" study areas). These study areas were visited at least once during every month of the year, excepting December and January. Observations of behavior were also made at a number of other localities in Oklahoma and Texas. In general, most of the conclusions regarding the behavior of the pupfish are a synopsis of observations from all the localities at which pupfish were observed. In the discussion below, the date and locality is given for specific examples of behavior.

Data from two seining trips (June 8 to 14 and July 4 to 8, 1969) provided the basis for an analysis of factors affecting the distribution of fishes within the range of _C_. _rubrofluviatilis_. A total of 59 localities were visited during the two seining trips; these sites were scattered as randomly as possible over the range of the pupfish. Collections were made in all of the major tributaries at widely scattered points. Besides the locality, data recorded at each collection site included general comments concerning turbidity, width and depth of the stream, vegetation, bottom type, speed of the current, and often the specific situation in which a particular seine haul was made (e.g., riffle, pool, etc.). A water sample was taken at each collection site and total dissolved solids content was measured with an electrical dissolved solids meter.

Seining was done with a 10-foot "common sense" seine. An attempt was made to sample all the different habitats at each collection site; these could be categorized as follows: (1) riffles, (2) pools, (3) the current of the mainstream, (4) coves more or less isolated from the mainstream, and (5) intermittent pools. Each species was enumerated and recorded according to the seine-haul(s) in which it was taken.

For each species, the number of individuals collected at each locality was corrected to a standard of five seine-hauls. This was done by using the number of seine-hauls in which the species was collected as the denominator of a fraction having five as the numerator, and the resulting fraction was multiplied by the total number of individuals of the species taken at the site.

These computations were done in an attempt to make the abundance of the various species comparable from one locality to the next, regardless of the relative abundance of the different habitats (i.e., riffle areas, pools, etc.). The basis for this method of handling the data is the assumption that individual seine-hauls containing a particular species can be equated with the "preferred" habitat of that species; then, by correcting for a standard number of seine-hauls containing the species, the preferred habitat is corrected to a standard value. This allows for a more accurate evaluation of the effects of environmental factors, such as the chemical nature of the water, on the abundance of a particular species.

Certain species were lumped together in counting the number of seine-hauls (the denominator of the fraction) on the basis of their having similar habits in terms of "preferred" habitat. The families Centrarchidae and Ictaluridae were considered individual entities; Carpiodes, Ictiobus, and Cyprinus were all lumped together as a single group; Pimephales vigilax and P. promelas were considered a single group. The number of seine hauls containing all other fishes were counted separately for the various species.

Data from 66 collections made by Dr. Hague Lindsey and Russel Bates in the summer of 1963 as part of the Oklahoma Biological Survey were used in conjunction with data gathered during this study in determining species associations (Table 3). Only those collections from within the range of the pupfish were used. These workers were very thorough, and I believe that their collections were representative of the fish population at the collection sites. All 59 collections made during the present study were used in determining species associations.

Ten collections taken from areas where the stream had dried to intermittent pools were excluded from consideration in the comparisons illustrated in Figures 12-18. These collections were excluded because intermittent pools offer no opportunity for influx or efflux of fishes in response to changing conditions; thus, collections from them may not be representative of the "voluntary" fish population at given points in time.

In figuring product-moment correlation coefficients for proportionate data, the angular transformations of the proportions were used as prescribed by Sokal and Rholf (1969).

Examination of the anterior two-thirds of the digestive tract of 50 specimens, ranging from 9 to 60 mm, TL, was used to assess the food habits of the pupfish. The localities and dates of capture of these fish were as follows: Oscar Creek, Jefferson Co., Oklahoma, 18 June 1967 (2 juveniles, 6 adult females, 7 males); a small tributary of North Fork of the Red River at T-1N, R-19W, S-18 & 19, Jackson Co., Oklahoma, 11 June 1969 (5 females, 4 males); Elm Creek at Highway 30 bridge, Harmon Co., Oklahoma, 12 June 1969 (4 females, 7 males); Prairie Dog Town Fork of the Red River, south of Hollis, Harmon Co., Oklahoma, 12 June 1969 (3 females, 5 males); North Fork of the Red River at Highway 83 bridge, Wheeler Co., Texas, 13 June 1969 (4 females, 3 males).

CHAPTER III

GENERAL ECOLOGY

Distribution and General Habitat

Figure 1 shows the distribution of *C. rubrofluviatilis* in the Red and Brazos rivers, based on records from the University of Oklahoma Museum of Zoology (UOMZ) and my collection localities. Pupfish were not found on the High Plains of Texas during this study, but were found in both river systems just east of the Caprock Escarpment, the eastern limit of the High Plains. The eastern-most extent of the range in Oklahoma is a small tributary of Red Creek near Oscar, Jefferson County (herein referred to as "Oscar Creek").

Much of the range is underlain by beds of salt with extensive areas of overlying gypsum ($CaSO_4$). Ground water percolating through salt and gypsum and surface water flowing over the beds of gypsum dissolve large quantities of minerals; thus many of the streams carry large quantities of dissolved solids. Also contributing to the high mineral content of the water are the extensive oil fields in certain areas from which brine is sometimes allowed to escape into the streams. Irelan and Mendieta (1964) give the chemical characteristics for the Brazos River and similar information can be obtained for the Red River from the U.S. Public Health Service (1964).

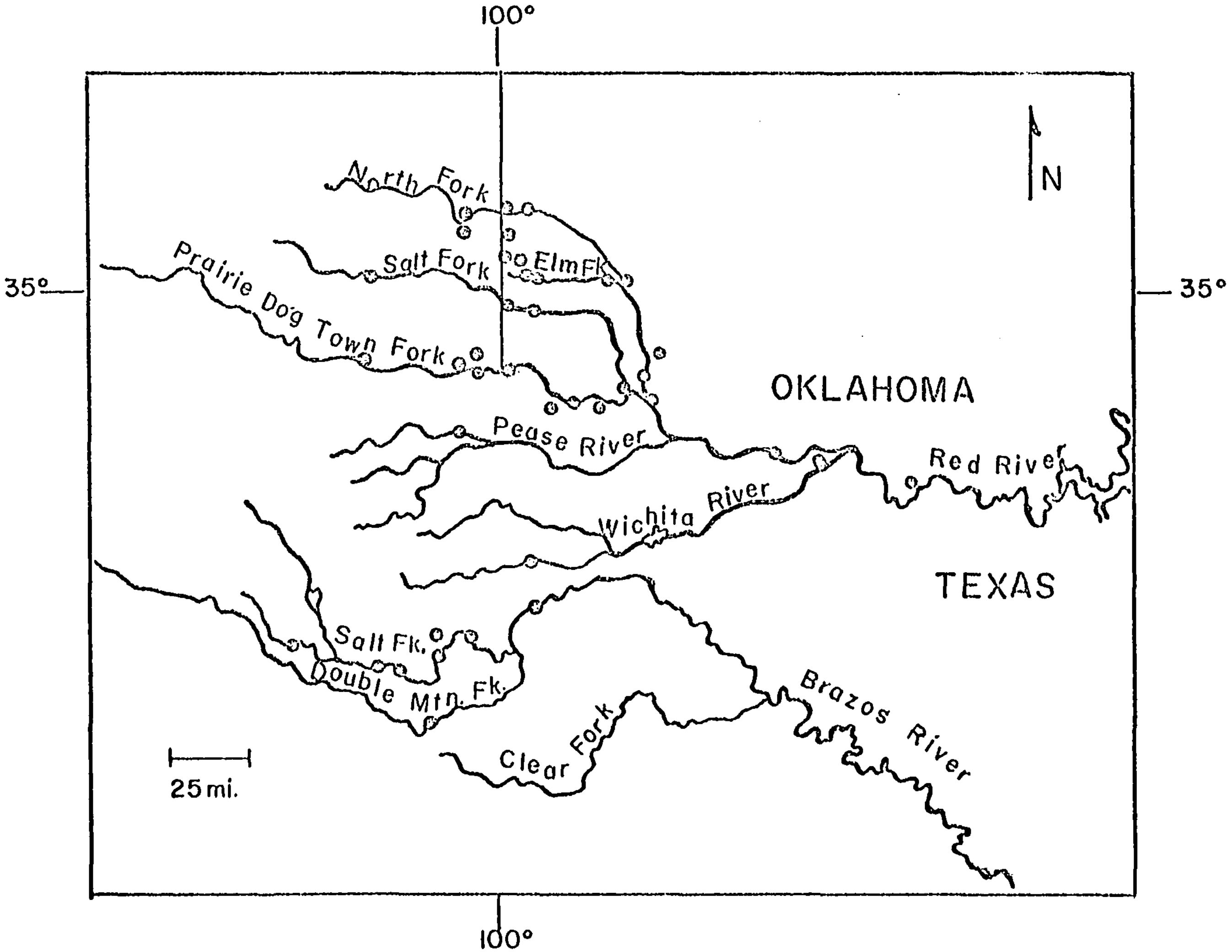

Figure 1. Distribution of the Red River pupfish in the Red and Brazos rivers of Oklahoma and Texas.

The rivers within the range of the Red River pupfish are shallow, low-gradient streams with sandy bottoms that are wide and flattened. They exhibit extensive fluctuations in water-level, becoming swollen and swift after periods of moderate to heavy rainfall, and receding to intermittent pools that may dry up during dry weather--especially in regions near the upper terminae of the streams. This results in striking fluctuations in the chemical content of the water, as described for the Brazos River by Irelan and Mendieta (1964):

> When rains produce runoff in a channel that has been dry, the first flow of water rapidly dissolves the saline residues in the sand and gravel and a surge of salty water moves down the stream. After this surge, the stream water usually contains much less dissolved material. If the stream has several tributaries, variations in the intensity of the rain and in the movement of the storm pattern will cause the water from different tributaries to reach the main stream at different times; thus, a series of individual slugs of salty water may move down the channel. After the flushouts, the stream water may be low in dissolved solids as long as the overland flow persists. However, the dissolved-solids content usually increases with time and with a decrease in streamflow, as a greater part of the flow is lost through evaporation and as the proportion contributed by ground water becomes larger.

Seasonal Changes in the Behavior of the Pupfish Population

Excluding long-term effects of gradual changes in photoperiod and the general temperature regime, the immediate temperature of the water is probably the major factor mediating the behavior of the pupfish population. In southwestern Oklahoma, winter air temperatures frequently varied from as low as 30 F at dawn to 60 F or 70 F in the afternoon. In the summer it can vary from 70 F at dawn to around 100 F in the afternoon. In shallow flowing water the temperature varied directly with the air temperature--lagging behind it only one or two degrees. These changes in water temperature were correlated with obvious changes in pupfish behavior.

At temperatures lower than 50 F in winter and early spring, pupfish were found primarily in deep quiet water. These were places of minimal current where they were not confronted with the problem of position maintenance that existed in the stronger current of shallower waters. When more or less isolated from the current, such areas also buffer abrupt temperature changes. Movement into the current was seldom observed at temperatures lower than 50 F.

In cold weather the temperature regime of the mainstream was quite different from that found in pools (ca. 2 to 40 ft, dia.) more or less isolated from the current. In regions affected by the current the temperature was relatively homogeneous from top to bottom, while in less affected areas there were varying degrees of thermal stratification over short vertical distances (e.g., 10 cm), especially when an ice-film covered the surface. Observation of the depths at which fish were found indicated selection for warmer temperatures. At near-freezing air temperatures when there was no activity in the mainstream, pupfish in isolated pools were often seen feeding and exhibiting other activities in the deepest water (30 to 50 cm) where the temperature was usually 40 F or above; this roughly corresponded with the temperature at which they became active in the mainstream.

There appeared to be age and sexual differences in degree of activity at lower temperatures. Females and juveniles seemed to be active earlier in the day and occurred in shallower areas than the larger males. Large numbers of small pupfish ($<$ 15 mm, TL) were frequently observed feeding actively in the shallowest of water at temperatures below 40 F.

Sexual differences in activity at low temperatures may be related to differences in cold tolerance. In the Childress area on 17 February 1968, 61 male pupfish and 40 females were found that apparently had been killed as a result of the consistently cold temperatures (below 32 F) of the previous week. Testing the sex ratio of the dead fish against a theoretical 1 : 1 ratio yielded a significant Chi-square value ($p < .05$). Comparison with the sex ratio (1.1♀ : 1♂) of 167 live fish collected on 10 and 11 February 1968 in the same area gave a highly significant Chi-square value ($p < .01$).

At water temperatures below 50 F pupfish were commonly found as large aggregations in deep (e.g., 50 cm) depressions of the mainstream. The following is an edited account of an aggregation of approximately 3000 pupfish on 11 February 1968. Numbers represent time and water temperature, respectively, for each note.

> 0700 (31 F): Aggregation compact; majority facing the current and resting motionlessly on the bottom except for an occasional low amplitude movement. Most movement seems aimed at maintaining position in the current. No feeding observed.
>
> 0925 (33 F): Some milling around occurring; portions of the aggregate moving in one direction and then in another as groups of small fish begin to leave the main mass and then return as if frightened. Larger fish remaining near the center of the mass in the deepest water. Movement slow and sluggish.
>
> 1030 (38 F): Aggregation dispersing considerably; fish tending to form schools that move over the bottom in troop-like fashion. Some foraging activity among schooling fish.

Some in shallower water, still deeper than 20 cm; majority of the larger fish remaining aggregated in deepest water.

1150 (46 F): Increased tendency for fish to swim off the bottom and to move as individuals rather than as groups. About 200 feeding on floating debris; many small fish at the water's edge in the shallowest water.

1220 (50 F): Much more widely dispersed; many fish at the surface and in midwater. Foraging has increased considerably. Fish behaving primarily as individuals, but schooling-up when startled. Groups of 200-300 small fish swimming around edge of pool in long columns. Larger males tend to remain near the bottom. Chafing occurring more commonly.

1245 (52 F): Small pieces of floating debris frequently accompanied downstream by several fish; after a distance of 10 to 20 ft from the pool, they desert the debris and return. There seems to be a strong tendency to remain in the pool. This was the maximum temperature for the day; no permanent emigration observed.

At higher temperatures fish began to emigrate from the pools and occurred in increasing abundance in the current. Emigration is apparently temperature dependent; it occurred at different times of the day depending on the water temperature. On 25 and 26 February 1968, movement from the deeper areas began at approximately 1200 when the water temperature was above 50 F in the Childress area. Later in the spring, emigration began much earlier and fish were found in the current at daylight. In February and March there seemed to be a significant movement of fish back into deeper areas shortly before nightfall; this may have been in response to the changing light intensity.

At summer temperatures, pupfish were distributed everywhere in Oscar Creek at all times of the day; however, there was a tendency for greater numbers of fish to occur in the shallows just after the drop in temperature from the mid-afternoon high (Fig. 2). As in the winter,

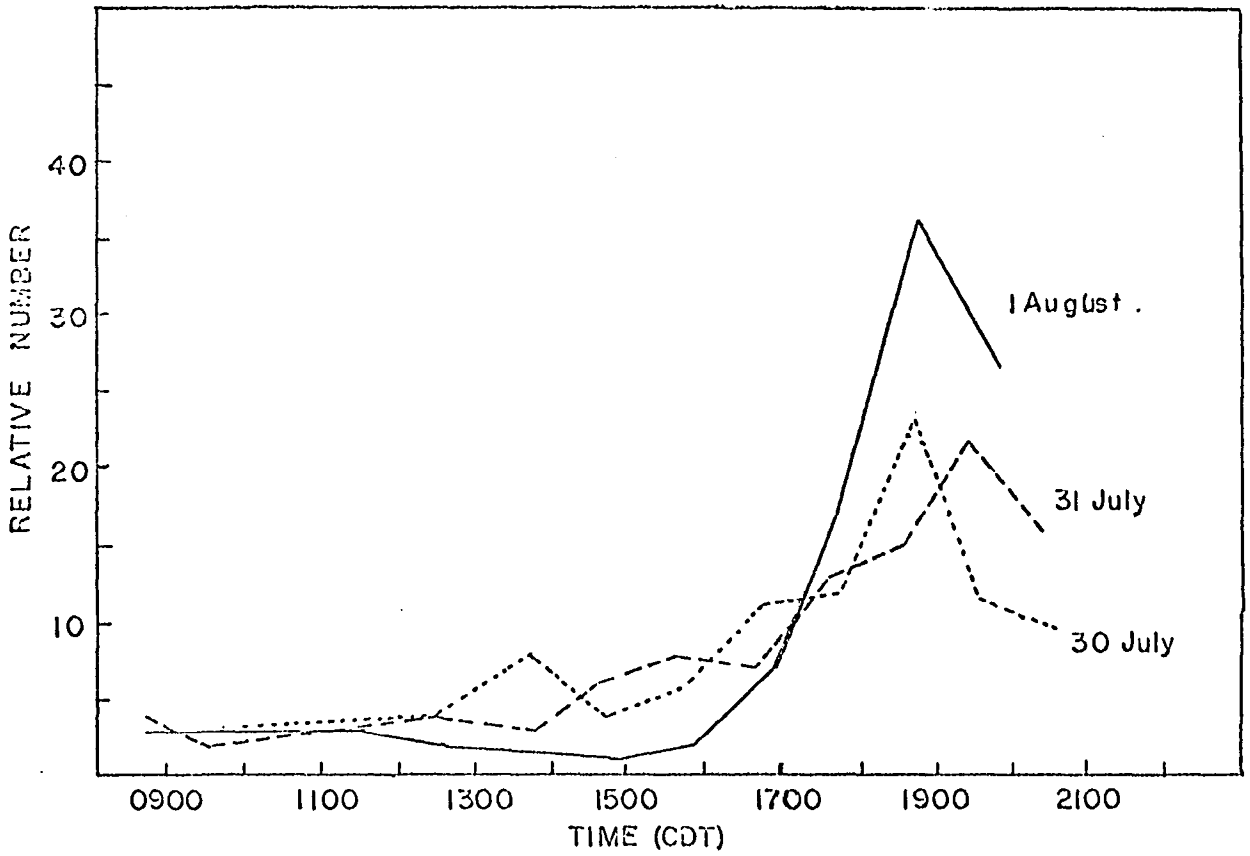

Figure 2. Relative number of pupfish counted in a shallow, 25-foot segment of Oscar Creek at different hours on three consecutive days in 1969. The increase later in the day represented movement from a deep area just upstream. Relative number is expressed as the percent of the number of fish counted during the day. Males in breeding territories are not considered.

there seemed to be a movement back to deeper water just before nightfall, but this was less pronounced in the summer.

As expected, feeding activity was minimal at low temperatures and maximal at higher temperatures. At temperatures less than 40 F pupfish tended to lie in dense aggregations on the bottom in deeper waters. They fed very little at these temperatures and aggression was minimal or nonexistent except that occasionally a movement of one individual inadvertently(?) displaced another. At temperatures of 40-50 F, they tended to move about in tight schools, periodically stopping to feed on the bottom. Once the schools stopped moving the fish then began to orient and feed as individuals. Feeding activity intensified with increased temperature, and at temperatures above 60 F they exhibited less tendency to move as schools and became more dispersed over the feeding areas. The increased dispersion seemed due to higher degrees of mobility and increased aggression at higher temperatures.

Based on visual observations of the spawning act, spawning begins in February and continues into November. The earliest spawning observed in this study occurred on 26 February 1968 in the Childress area. The latest spawning was observed on 1 November 1969 in Oscar Creek. Spawning occurs in territories established by the males in shallow water. Breeding territoriality is discussed in some detail in a later section of the paper.

At the beginning of the reproductive season, males consistently began defending territories when the water temperature approached 55 F (Table 1), suggesting that the initiation of territoriality is temperature dependent. Later in the season, territories were defended from dawn until

Table 1. Time of day and temperature at which male pupfish began defending breeding territories in the Prairie Dog Town Fork of the Red River near Childress, Texas, 1968.

Date	Time	Corresponding temp. (F)	Situation
10 II 68	1630	56	mainstream
17 II 68	no territoriality	max. temp. = 37	mainstream
25 II 68	1600	56	isolated pool
2 III 68	no territoriality	max. temp. = 59	mainstream
9 III 68	1030	54	isolated pool
16 III 68	1100	60	mainstream
17 III 68	1230 1245	59 56	mainstream isolated pool
24 III 68	1100	52	isolated pool
25 III 68	1015	55	isolated pool
29 III 68	0649	58	isolated pool
30 III 68	0642	59	mainstream
5 IV 68	1145	58	isolated pool

dark. At temperatures exceeding 90-95 F, many males restricted their activity to defence of small feeding areas near the center of the breeding territory or else deserted their territories altogether. These desertions were reflected in the number of territorial males seen in certain areas. In the summer the number of territorial males counted in a shallow segment of the stream in Oscar Creek was maximal in the morning and minimal in early afternoon (Fig. 3). Later in the afternoon their numbers gradually increased as they returned to areas occupied earlier in the day. The shallowest waters represent the most rapidly warming areas, thus the males were probably migrating to deeper, cooler water. Males occupying territories in the shallowest of water were the first to leave while those in deeper areas tended to defend their territories throughout the day. Movement back to the deserted areas was correlated with a drop in temperature from the mid-afternoon high. Occasionally a male deserted his territory, moved into deeper water, and returned in several minutes to resume territorial activities; this was seen most commonly at temperatures above 90 F.

In winter and early spring the males deserted their territories at nightfall and moved to deeper water; then, at temperatures near 50 F the following day, they returned to the shallows and resumed territoriality. For the following reasons, territorial males apparently tend to return to the same territories day after day: (1) similar sized males were seen at the same site on consecutive days, (2) behaviorally different males in terms of levels of activity were consistently seen at the same site, and (3) the size and shape of the territories in

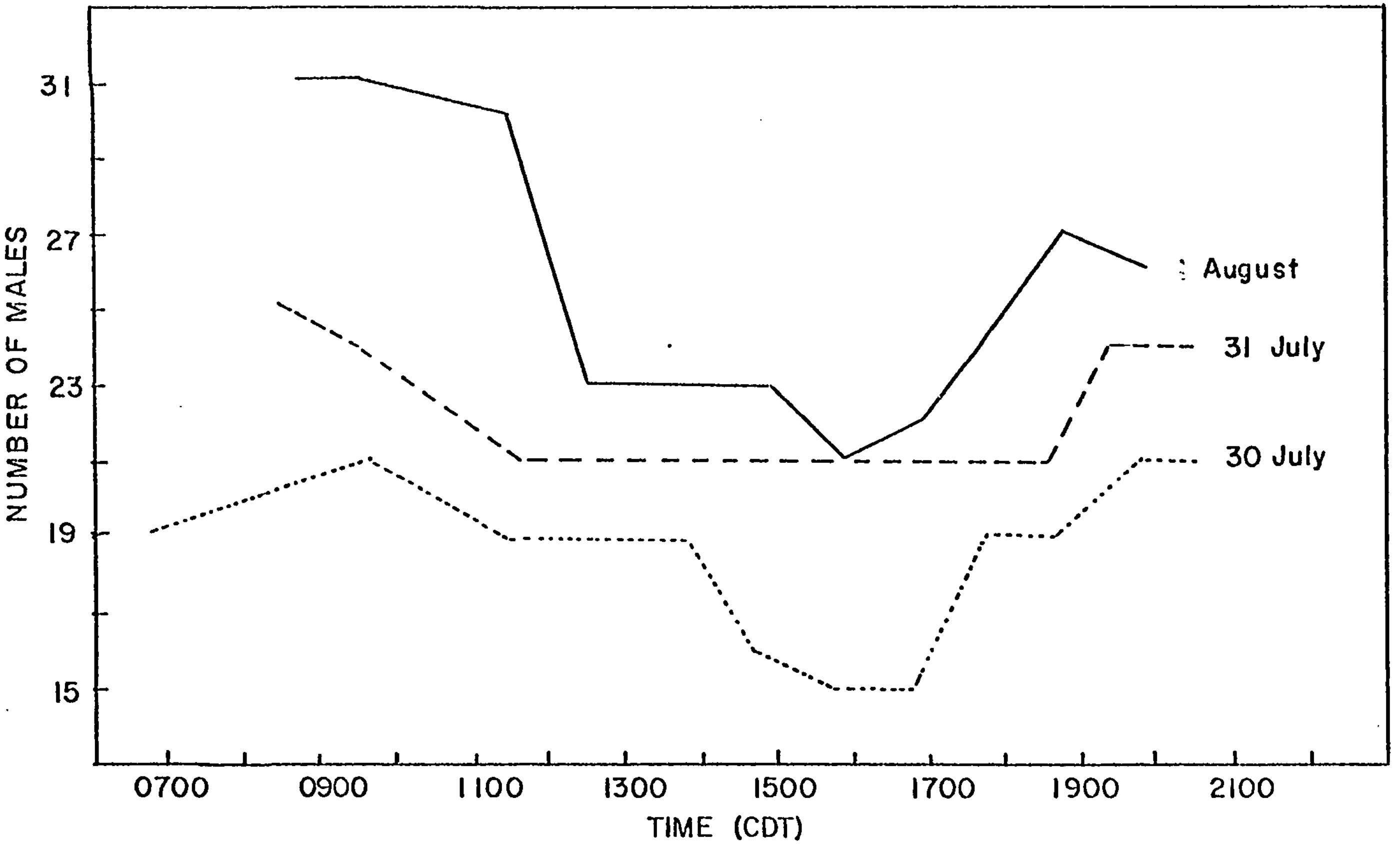

Figure 3. Number of males defending breeding territories in a shallow 25-foot segment of Oscar Creek at different times on three consecutive days in 1969.

particular areas were fairly consistent from one day to the next. In the summer, there was more of a tendency for males to rest at night within their territories. Cox (1966) found that one *C. macularius* male maintained the same territory for at least 28 days.

Spawning occurred in daylight hours at temperatures ranging from 55 to 93 F. In the winter, it usually began in the afternoon when the temperature neared 55 F, while in warmer months it began before sunrise and continued until nightfall or until the temperature neared the maximum for spawning. This resulted in a shift from primarily afternoon spawning early in the reproductive season to day-long spawning at the moderate temperatures of spring to primarily morning spawning in the summer. In the summer, spawning reached a peak at mid-morning (water temp. = 75 to 85 F) and then slowed until completely over around noon, when the water temperature approached 90 F (Fig. 4). In June and July of 1968, spawning occurred regularly in aquaria at temperatures ranging from 60 to 86 F.

Spawning occurred sporadically late in the reproductive season. In October and November only a few males could be found defending territories when water temperatures were above 60 F. Earlier in the year they were found in almost every available area at comparable temperatures. Most of the territorial males seen in October and November were less than 30 mm, TL. Males as large as 50 mm, TL, were commonly seen, but they usually were not defending territories. Based on the sizes of the participants, most of the breeding population were almost certainly young-of-year individuals.

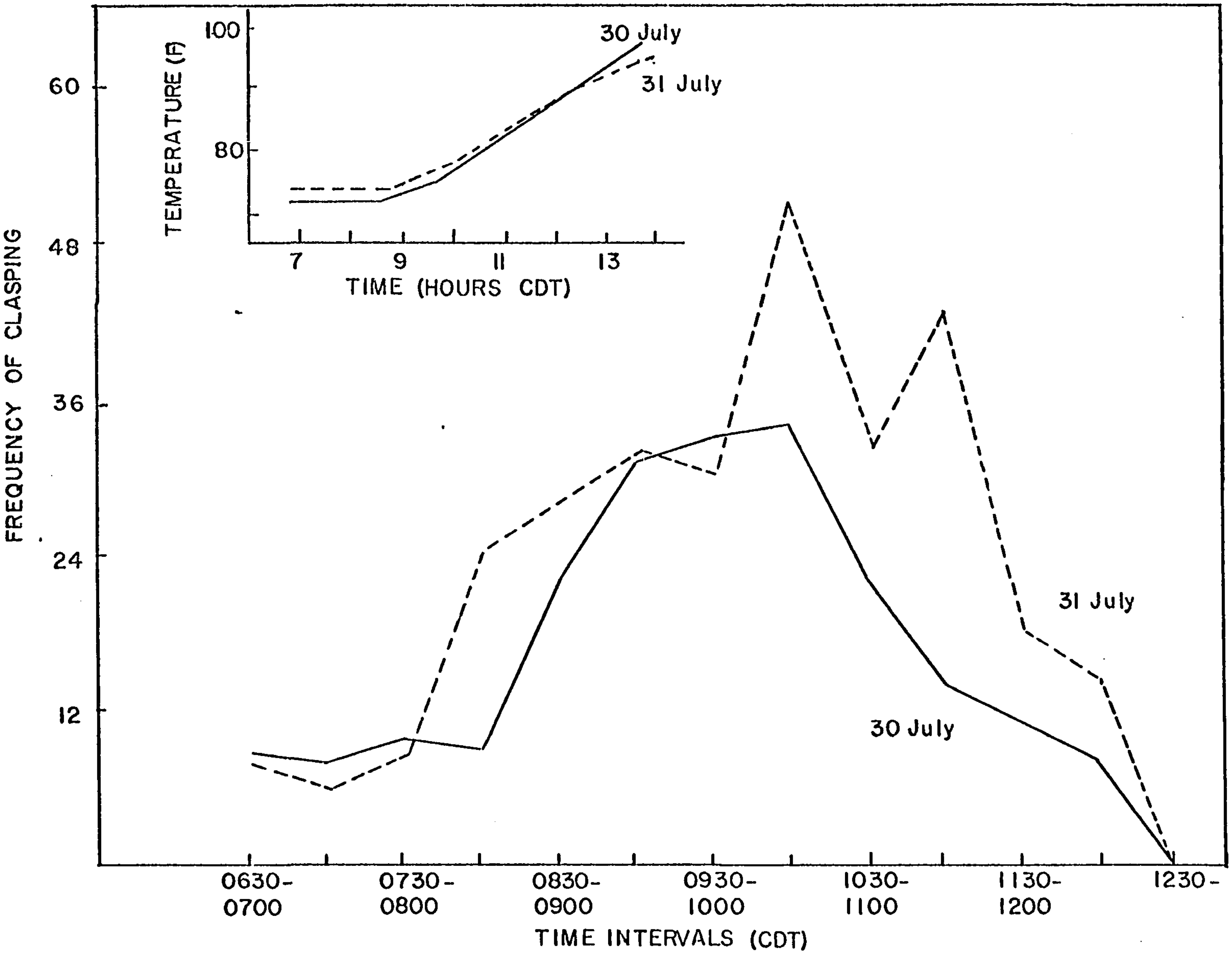

Figure 4. Frequency of spawning in 30-minute intervals in a group of 21 breeding males on 30 July 1969 and 25 males on 31 July 1969 in a 25-foot segment of Oscar Creek. Frequency of spawning is expressed as the number of courtship sequences in which clasping occurred.

Schooling and Aggregative Behavior

Red River pupfish formed schools similar to those described by Barlow (1961) for C. *macularius*. They consisted of several to hundreds of individuals spread out primarily in the horizontal plane, especially when swimming against the current. The size of the fish was fairly homogeneous in a particular school, probably because the larger fish swim faster than the smaller, thus segregating the size groups. In the current, schools tended to swim near the bottom, moving around rather than over vertical irregularities. In quieter water they were more independent of the bottom and were frequently seen swimming near the surface; however, pupfish in general seem to have a propensity for the bottom.

In early spring there seemed to be a general upstream migration in the Childress area with the pupfish leaving the deeper pools in schools. Numerous schools were observed departing from pools into the shallow waters of the mainstream, and almost without exception, they moved upstream. Upstream migration was not observed in late spring or summer.

The migration may function in at least three ways: (1) dispersal of breeding males, (2) increased male-female encounters, and (3) more efficient utilization of food resources. Individual males seemed more successful at consummating courtship with spawning in areas where the density of territorial males was relatively low. There was much antagonism between neighboring males, especially at high densities where the individual territories were restricted in size. In such situations there was intense competition for receptive females, resulting in a high incidence of interference once a particular male began his courtship antics. Assem (1967) described similar effects for the three-spined stickleback, Gasterosteus aculeatus. Such intrusions often resulted in aborted courtship.

As schooling females moved upstream they frequently encountered several males in succession in passing through the individual territories. This often led to courtship and spawning. One possible advantage is that females have the opportunity to spawn in a variety of areas, and at least some of the eggs may incubate in favorable situations.

The migration probably facilitates the discovery and utilization of feeding areas upstream. This might be important in view of the increasingly higher temperatures of spring and summer and the correspondingly higher nutritional demands.

Pupfish also moved as schools over shorter distances in a more localized type of movement. Often members of feeding aggregations suddenly "schooled up", moved a short distance, then stopped and resumed feeding. At higher temperatures these schools consisted largely of females and juveniles, since a large portion of the males was confined to reproductive territories. A second type of local movement, most pronounced in colder weather, took place when the population moved between night-time "resting areas" (usually deeper pools) and preferred day-time feeding areas. As the temperature rose during the day, small schools began to radiate from the resting areas. Local movement, like the migrations, effects increased male-female encounters, and perhaps greater efficiency in the discovery and exploitation of food concentrations. Feeding movements are strongly allelomimetic, and the initiation of feeding in one member of the school often stimulates feeding in others.

Schooling up often occurred as flight to deeper water or cover spread through the aggregation. Although this is probably due in part to the summation of individual fright responses, it is also allelomimetic in that fish not directly affected by the fright stimulus may rush into deeper water along with the initially frightened individuals. On one occasion this behavior was elicited in response to a low-flying vulture that cast its shadow over the water.

Fright frequently led to aggregation in deep water or cover material after which the fish slowly began moving back to feeding areas. Another form of aggregative behavior was the resting aggregation. Periods of low temperatures (less than 40 F) and night were times of relative inactivity and pupfish tended to aggregate in deeper areas of minimum current. At low temperatures, some members of resting aggregations were found buried in the bottom material, as described for _C_. _macularius_ by Cox (1966), while others were resting on the surface of the bottom. Because of the current, individuals resting on the substrate in the mainstream were more active in maintaining position than buried individuals.

Feeding Habits

Excluding males in breeding territories, pupfish fed primarily in aggregations of from several to hundreds of fish. Large aggregations occurred frequently in shallow coves and in other areas of relatively quiet water where they sometimes covered the entire bottom, but smaller aggregations (e.g., fewer than 100 fish) were seen in areas of slight to moderate current in the mainstream of the river. In these aggregations the fish oriented and behaved as individuals, moving in any direction from one portion of the aggregation to another. Feeding activities usually consisted primarily of nipping and digging, with other feeding activities (described in section on motor patterns) occurring at lesser frequencies. Some feeding occurred at the surface, especially if there were floating algal mats or debris, but most was confined to the bottom.

The major items of the intestinal contents of the 50 pupfish examined in this study were as follows, in decreasing order of frequency of occurrence: diatoms (38), filamentous green algae (20), dipteran larvae (10), nematodes (8), copepods (6), fungal spores (5), and adult insect remains (4). Miscellaneous items included acanthocephalans, plant fibers, root tips, and vascular plant seeds. Forty-one fish contained plant material and 20 contained animal material. The nematodes and acanthocephalans could have been parasites, but this was not determined. Sand occurred in 44 of the intestines and usually constituted the major bulk of the intestinal content. The following are arranged in order of decreasing bulk in the intestines: diatoms, filamentous green algae, and animal material. Plant material formed by far the major bulk of the food items found. An occasional pupfish was found with the gut engorged with filamentous algae.

The high incidence of sand corroborates behavioral evidence that Red River pupfish are primarily bottom-feeders, but their behavior and the occurrence of ants and winged insects show that some foods may be taken from the surface film. Feeding was observed at the surface, on the bottom and in midwater. They were occasionally seen feeding on items too large to be ingested whole such as grasshoppers and dead fish. This was done by tearing off pieces of the item with a sharp wrenching movement.

The intestinal contents and behavioral evidence indicate that Red River pupfish have very generalized food habits. Although primarily herbivorous, they are opportunistic and will ingest animal material when it is available. Kaill (1967) reported that three cyprinodontids (*C. variegatus*, *Jordanella*, and *Floridichthys*) are primarily herbivorous, and several workers reported that various species of *Cyprinodon* are completely or primarily herbivorous (*C. baconi*, Breder, 1934; *C. variegatus*, Hildebrand and Shroeder, 1928; and *C. macularius*, Cox, 1966, and Miller and Miller, 1942). Others reported that animal material constitutes the bulk of the foods: Kennedy (1916) found dipteran larvae the major food item in 14 *C. radiosus*, and Gilbert (1893) and Leser and Deacon (1968, unpublished report to National Park Service) found that animal material constituted the bulk of the diet for *C. nevadensis*. Martin (1968) found that 67 of 100 *C. variegatus* (a close relative of *C. rubrofluviatilis*) contained plant material (primarily algae) and 17 contained animal material, but he concluded "...there was little evidence of digestion of algae. Samples taken from the posterior-most part of the intestine contained apparently living [algal] cells.... On one occasion a sample was taken and cultured. Growth was exhibited." The occurrence of living plant cells in the posterior region of the digestive tract does not mean, however, that there is no digestion of significant amounts in the anterior region of the gut. Martin (*l.c.*) suggested that the large amount of vegetable matter is ingested accidentally in the process of obtaining animal food, and inferred that pupfish are inefficient in their foraging activity.

I observed pupfish feeding on filamentous algae and mats of non-filamentous algae in such a way that they apparently were not searching for animals within the mass of plant material--they wrenched or pulled repeatedly at one spot in the algal mass for sustained periods and did not exhibit the searching behavior that would be expected if they were attempting to pick animals from the algae. In view of the large amount of energy expended in such activity, it would seem a case of extreme negative selection if they were not obtaining nutritive value. There is evidence that certain fish can digest diatoms but are incapable of digesting green or blue-green algae due to the apparent absence of cellulase (Barrington, 1957), suggesting that diatoms may be a major portion of the pupfish diet, even if other algae are not digested. Peristaltic action aided by the sand usually found in the gut may function to rupture algal cells so as to render the protoplast available for digestion.

It is possible that, as Minckley and Itskowitz (1967) suggested for an unidentified Mexican *Cyprinodon*, *C. rubrofluviatilis* obtains nutriment from the organic sediments of the substrate material, they stated: "*Cyprinodon* 'A' inhabits warm saline pools.... In such habitats, devoid of animiculae and rigorous at best, summer food of

C. 'A' consists of digestible components of the sediment, which it devours voraciously."

Because of the difficulty in making a quantitative study of food items as small as diatoms, an alternative method involving counts of the number of feeding movements per unit time was used to determine feeding periodicity. In the summer, feeding activity in females increased gradually until about 1400 when it reached a plateau; it remained high until just before nightfall when feeding showed a decided decline (Fig. 5). In general, it seemed that females fed more actively than territorial males (Fig. 5). Territorial males fed very little until around 1300 when there was an increase that was roughly commensurate with the cessation of spawning activity. Like the females, feeding activity in territorial males reached a maximum at about 1400 and remained high until just before nightfall when there was a decline in activity (Fig. 5).

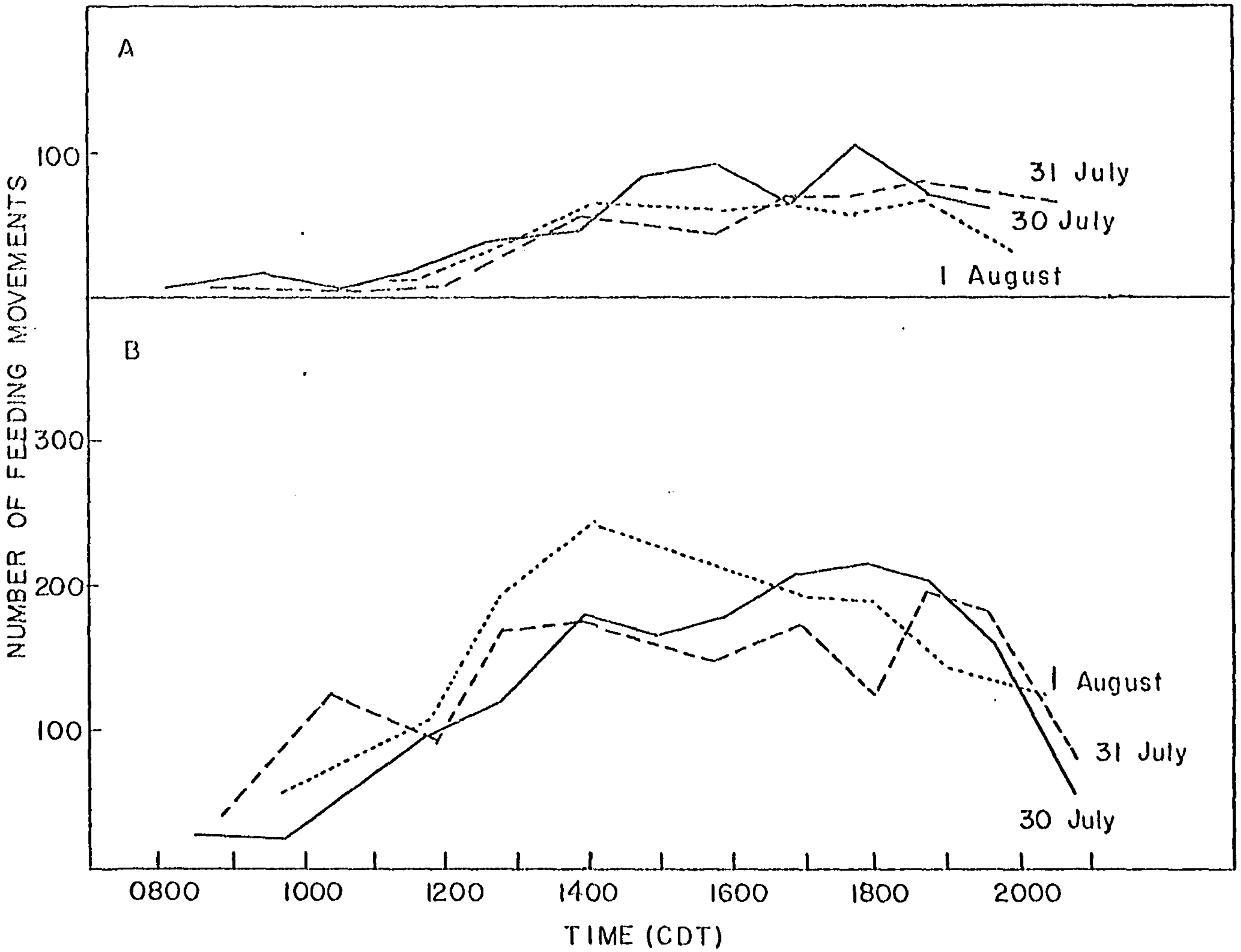

Figure 5. Periodicity of feeding in Red River pupfish on three consecutive days in 1969. A = males in breeding territories; B = females. Periodicity is expressed as the total number of feeding movements (nipping) counted in one-minute intervals for five individuals at different times of the day.

CHAPTER IV

BEHAVIOR

Motor Patterns

The following is an annotated list of behavioral motor patterns that were seen in the Red River pupfish. This represents an attempt to obtain a complete ethogram for those behavioral categories that are considered. To date, there are no publications which describe the complete behavioral repertory for any species of *Cyprinodon*; thus some of the names are of my own choosing, but many were taken from various authors, e.g., Barlow (1961), Kaill (1967), and Foster (1967). In the following list, an asterisk is placed beside those names which are new or else have not been previously applied to behavior in the genus *Cyprinodon*.

Feeding Motor Patterns

Wrenching. The fish grasps a food object in its mouth, and with a sharp lateral jerk of the head, tears a particle of food away. Median fins are partially spread during this movement. This is done when feeding on objects too large to swallow whole (e.g., dead fish, grasshoppers, etc.), and sometimes when apparently feeding on periphyton.

Nipping. The mouth is opened, pressed against the substrate and quickly closed, taking in a particle of food in the process, often along with some substrate material (e.g., sand). Nipping is done with the head tilted down when feeding on the bottom, but depending on the situation, it may be done from the horizontal position or with the head tilted up. The nipping movement may grade slowly or quickly from hovering through tilting, or the fish may tilt, hold position momentarily, and then nip. After nipping, the fish may return to the horizontal, hover briefly, and then tilt and nip at the same site or slightly to wne side. Often the tilt position is maintained while the fish nips successively at the substrate.

Fish feeding within the territory of a dominant male often nipped in a "furtive" manner, in that it was most likely to occur with the male facing in the opposite direction. In these instances, the nip was a jerky movement with a quick return to the horizontal.

Digging. This could be interpreted as exaggerated nipping during which the head is driven forcibly into the substrate material, sometimes up to the eye. The head is withdrawn from the substrate by a backward movement of the body as the fish returns to the horizontal. Sand grains are usually expelled forcibly from the mouth, often after momentarily "mouthing" the material, and smaller sand grains may trickle from the opercular openings. The fish may dig only once, or it may move a short distance and dig again. Tinbergen (1951) described a similar activity for the three-spined stickleback, Gasterosteus aculeatus, in which it is an agonistic response. In pupfish, it is apparently a feeding activity that is not related to agonistic behavior. Digging may be a common behavior among cyprinodontids, as I have seen it in

C. elegans and _Fundulus zebrinus_ (= _kansae_), and it has been described for _C. macularius_ (Barlow, 1961), _Jordanella_, _Floridichthys_, and _C. variegatus_ (Kaill, 1967).

Plowing. The fish tilts, presses the mouth to the substrate as if to nip, and then quickly settles tothe bottom and moves slowly forward with rapid lateral undulations of the body. Bottom material is thrown to the sides. When plowing is terminated the pectorals cease resisting forward movement and the fish moves slightly forward and upward from the substrate. Then it usually turns around or backs up and nips or digs in the resulting excavation; sometimes several plowing bouts occur in succession with no interrupting feeding bouts. I have also seen plowing in _C. variegatus_ and _C. elegans_; it was described for _C. macularius_ by Barlow (_l.c._). W. L. Minckley (pers. comm.) is preparing a paper concerned with plowing in several _Cyprinodon_.

_Fanning_*. This was observed on only one occasion. It seemed very similar to parental fanning in _Jordanella_ as described by Mertz and Barlow (1966). The behavior was essentially the same as plowing, except that it was done about one centimeter off the bottom and the beating of the caudal and pectoral fins stirred up only the loose debris on the bottom of the aquarium. At the end of each fanning bout the fish hovered and seemed to feed on suspended particles. After feeding, the fish tilted head downward and, without touching the substrate, began another fanning bout. Fanning bouts were repeated six times during the observations. The undulations were of lower frequency than plowing, but of similar amplitude. Fanning was very distinct from hovering in that the undulations were lower in frequency and of greater amplitude than in hovering. Fanning in feeding context has been described for Jordanella (Kaill, l.c.; Mertz and Barlow, l.c.; and Foster, l.c.), but it has not been reported for any other cyprinodontine. Similar behavior occurs in gasterosteids (Reisman, 1963).

Aspiration*. Food particles are sucked with a current of water into the mouth in the direction of a negative pressure created by expansion of the pharyngeal cavity. Suspended particles or particles on the bottom may be ingested in this manner. This is common among fishes.

Pulling*. The fish grasps an object (e.g., a piece of filamentous algae or a root filament of an aquatic plant) with the mouth and then backs up, pulling the object along. Wrenching is sometimes employed as the fish attempts to break off a piece of the object.

Reproductive Motor Patterns

Driving*. Includes herding and steering. The male swims parallel, slightly postero- or antero-laterally, with the female, keeping between her and the territorial boundary.

Herding*. The male moves so as to contain the female's flight within the boundary of his territory. The female usually swims rapidly, making evasive turns to the side opposite the male. The male makes compensating turns, usually from a slightly posterior position, which places him between her and the territorial boundary. The pair usually chase back and forth across the male's territory during this phase of courtship.

Steering*. The male swims next to the female, his body between her and the territorial boundary, as she swims in an arc toward the center of the territory. In midwater they descend to the bottom in an ever-tightening spiral (especially in aquaria), or in a wide arc. When initiated on the bottom, steering leads to increasingly tighter spirals or it is very brief. The male's median fins are folded or partly raised in this maneuver. Steering may develop from or evolve into herding and it is sometimes hard to distinguish between the two. The progression from herding to steering leads somewhat predictably to further courtship, while the progression from steering to herding usually leads to escape by the female.

Looping. The male moves rapidly to the left and right in front of the female, his path tracing tight figure-eights or ovals elongated perpendicularly to her median axis. His median fins are folded or submaximally spread. Looping is done just below the level of the female if she is high off the bottom; if she is on the bottom it tends to be done in a slightly oblique plane inclined down toward her.

Zigzagging*. The male, from in front of and slightly below the female, moves rapidly to the left and right while progressing forward. This is seen most frequently when the female is moving rapidly on a straight-line course through the male's area. The presence of the male apparently affects the path of the female even though he usually is below her level and not physically obstructing her path. Sometimes she makes "dodging" movements where, for instance, if the male is moving to the left she veers to the right causing the male to make a compensating move to the right whereupon she veers to the left, and so on; this results in pronounced zigzagging by the male that apparently results from his tendency to remain in front of her. The female frequently swims straight through the territory, in apparent indifference to the antics of the male. Males were observed zigzagging in front of whole schools of females moving through the territories. Looping may simply represent "stationary zigzagging".

Contacting. This usually occurs when the pair is situated on the bottom, and includes all body contact that occurs prior to clasping. The male may shift excitedly from one side of the female to the other by moving laterally over her back. He frequently contacts her anteriorly with his snout and more posteriorly with his trunk as he attempts to initiate sidling. Interruptions often occur as the female moves forward, with the male attempting to keep beside her. Interruptions may also occur as the male briefly stops contacting to swim quickly around the front of the female to reapproach her from behind on the opposite side, or as he swims in a tight circle to reapproach her from behind on the same side. Contacting eventually evolves into and includes sidling.

Sidling. Usually the male initially contacts the female anteriorly with his snout and then swings his trunk against her, utilizing a sculling action of the caudal fin which is bent away from her toward the end of the maneuver. The male's dorsal fin is partially folded during sidling. The female may tilt and nip during sidling or she may remain passive or move forward several centimeters.

S-shaping. During sidling the male's body is thrown into an S-shape, usually with the caudal fin curved away from the female and the head turned towards her. As his trunk contacts her, the female either forms a complementary S-shape so that she fits into the curve of his body or else she may break contact by moving forward several centimeters, after which the male may initiate another sidling and S-shaping attempt. The median fin is maximally or submaximally spread during S-shaping. S-shaping is common among cyprinodontids (Foster, 1967).

Wrapping. During sidling and S-shaping the male's anal fin is spread and wrapped around the female's venter. From observations of aquarium fish it sometimes seemed that the anal fin was formed into a crude cup just beneath the female's vent (as reported by Barlow, 1961, for *C. macularius*), while at other times it was tightly wrapped just posterior to the vent. This is difficult to observe and it is not clear as to which is the usual case; it may be dependent on the relative sizes of the male and female. Wrapping in *C. rubrofluviatilis* is similar to that described for *C. macularius* by Cox (1966) and Barlow (1961) while it differs significantly from that of *Fundulus heteroclitus* (Newman, 1907) and *C. variegatus* (Kaill, 1967) in which both the dorsal fin and the anal fin are wrapped around the female. In view of their close taxonomic relationship (R. K. Liu, pers. comm.) this difference between *rubrofluviatilis* and *variegatus* was surprising; it may be due to experiential factors resulting from differences in the usual habitat of the two species.

Clasping. Includes S-shaping and wrapping. As clasping proceeds the male tends to move upward slightly on the anterior region of the female.

Vibrating. After clasping is effected the pectorals and caudal fin of the two fish vibrate rapidly, imparting a quivering appearance to the pair. The vibrations seem to begin rather slowly and then rapidly increase in frequency. This phase may be very brief, or it may be prolonged for several seconds. Vibrating is usually terminated at its peak by jerking, after which the clasp is broken. On one occasion the male left the female during this phase and the female continued vibrating in his absence with her body bent into an S-shape.

Jerking. After Barlow (1961): "While still S-shaped the head is jerked toward the side opposite that to which it is already directed, thus initiating a wave of contraction which passes down the body reversing the direction of the S-shape." In *C. macularius* jerking occurs in both sexes (Barlow, 1961, and Cox, 1966), while in *C. rubrofluviatilis* it is exhibited only by the female. The latter is also true of *C. variegatus*, *Jordanella*, and *Floridichthys* (Kaill, 1967). Cox (1966) reported that an egg is extruded about 50 per cent of the time after jerking in *C. macularius*.

Reproductive Nipping. Nipping in reproductive context is essentially the same as that described for feeding; at times it resembles digging more than nipping. The female nips the substrate while the male is contacting prior to and sometimes during the initiation of clasping. She may nip only once during a spawning sequence, or she may nip several times. Barlow (1961) described reproductive nipping for *C. macularius* and Foster (1967) reported that it occurs in *C. variegatus*, and many but not all, cyprinodontids. Reproductive nipping occurs in a quick jerky movement rather than in the slow, deliberate manner frequently seen in feeding fish. Nipping occasionally occurs as the female is approached by a breeding male.

Settling*. After nipping the female quickly drops her venter toward the bottom in a manner clearly different from the return to the horizontal usually seen in feeding fish. This is similar to the return to the horizontal that was often seen in feeding context as a fish nipped "furtively" at the substrate near a territorial male.

Guarding*. After the female has descended to the substrate, the male may dart around her in a tight circle, stop, and post. Then he may charge any fish, male or female, attracted by the courtship behavior. This does not often occur during courtship, but seems especially likely if the female descends to a spot near the territory of a male that has had frequent encounters with the courting male. If a threatening male is nearby, the courting male may swim in circles between the intruder and the female. Guarding should perhaps be listed as agonistic rather than courtship, since it does not seem to elicit any response from the female, and is apparently aimed at warding off intruders. In fact, if the male is slow to resume courtship the female may leave his territory.

Agonistic Motor Patterns

Most of the agonistic motor patterns described below occurred more frequently and in more stereotyped form in males defending breeding territories. Certain of the more routine motor patterns, such as tail-beating, chasing, and fleeing were also seen in females and non-breeding males, while other behaviors were not seen in these fish, e.g., ramming, escorting, and mouthfighting. Except where otherwise noted, the following motor patterns are described as they occurred in males defending breeding territories.

Patrolling. The male swims rapidly back and forth over his territory in a manner suggestive of investigative behavior. Forays across the territory sometimes occur continuously over long periods of time with the fish repeatedly crisscrossing over the area. This behavior is similar to that described for *C. macularius* (Barlow, 1961) and *C. variegatus* (Kaill, 1967).

Posting. The male hovers near the center of his territory, pivoting slightly to the left and right, giving the appearance of being on the alert for intruders. The pectoral fins flicker back and forth, being quite conspicuous because of their bright color.

Facing. After Barlow (*l.c.*): "Males approach one another head-on and usually pause momentarily face to face about one body length apart. The median fins are spread." In Red River pupfish, the body is usually bent into a sigmoid shape during these encounters. Facing was rarely seen in Red River pupfish. It seems to occur primarily in low intensity encounters.

Eyeing. After Barlow (l.c.): "Two males stand momentarily eye to eye facing in opposite directions, bodies parallel, and about one-half body length separating them." In Red River pupfish the body is bent in a slight sigmoid shape, head directed toward the opponent and caudal fin directed away. Fins are spread as in lateral display.

Lateral Display. A challenge display during which all fins are spread maximally and the pectoral and pelvic fins are pointed downward so that they extend below the ventral line of the profile, flat surfaces parallel with the sides of the body. The effect of this is that the fish presents his largest aspect to the opponent. Encounters may be terminated after lateral display or they may evolve into arching or escorting. Arching represents lateral display at highest intensity.

Arching. Two males usually orient head to tail with the body bent into a C-shape, concave side toward the opponent. The median and pelvic fins are spread maximally with the posterior edge of the dorsal cupped slightly toward the opponent. The pectoral on the side away from the opponent is pointed downward, while the other is often pointed more or less horizontally toward the opponent. At highest intensities, the beats of the pectoral and caudal fins impart a quivering appearance to the antagonists. In areas of moderate current the two fish tend to move in slow circles while in the arched position, presumably due to the effects of the current and attempts of the males to prevent being carried downstream. Sometimes the two males orient head to head, but this is unstable and they usually shift into the head to tail orientation. Another variation is where one male is arching and the other is oriented perpendicularly to it with the body straight and the snout directed into the concavity of the arching male. In these instances the arching male was much smaller than the other.

Swiping*. While eyeing, one male makes one to three quick "swipes" at the other, returning each time to the eyeing position; the second male remains stationary during swiping. This is rarely seen.

*Butting**. While eyeing, the two males butt each other one to several times in the head region using sidewise beats of the head while maintaining position.

Charging. The median fins are folded, the mouth is opened, and the fish darts at high speed toward another fish.

Chasing. After an initial charge the aggressor pursues the other fish more or less doggedly.

*Ramming**. The charging fish rams into the opponent at high speed. This was observed a number of times, but only in situations where one male rammed into the side of another that was clasping with a female.

Circling. After Barlow (1961): "From the arched position two fish often start to circle at high speed while maintaining the head to tail orientation. It seems that each fish attempts simultaneously to charge into the flank of the other and to dodge the charge of the opponent by turning."

Mouth-fighting. This was seen on numerous occasions in nature, but was never observed in laboratory aquaria. While circling, one or both combatants somehow manage to grasp the opponent's upper or lower jaw in its mouth. Then the two flip and jerk violently over the bottom of the area. Mouth-fighting was usually very brief, but sometimes lasted for several seconds. Newman (1907) reported mouthfighting in *C. variegatus* and *Fundulus heteroclitus* and a type of mouthfighting was described by Baerends and Baerends van Roon (1950) for cichlids.

*Facing away**. A fish turns to face the other way at the approach and/or challenge of a territorial male. Large males and other territorial males move slowly away from the challenger after this, while other intruders usually flee rapidly. Of course, "facing away" is a necessary prerequisite to any type of flight; however, it does seem to have an inhibiting effect on the attacking fish. Although the attacker may follow the intruder after it has faced away, it usually does not "speed up" the chase; in many instances it seemed as if the speed of the intruder determined the speed of the chase. The inhibiting effect may result from the fact that in facing away the tail is turned toward the opponent, and the tail is the "instrument" used in delivering blows during tail-beating. The inhibiting effect of facing away has apparently been exploited by other Cyprinodontinae, perhaps explaining how "T-circling" serves to hold the territorial male in abeyance (see below).

<u>Escorting</u>*. This occurs when the male's territory is invaded by a large male intruder. The resident swims aggressively toward the interloper, usually causing it to face away; then while maintaining the stiff posture of lateral display, the challenger follows from behind or slightly postero-laterally as the intruder moves away. The intruder sometimes moves slowly away as if reluctant to leave the territory. The pectoral fins of the challenger vibrate rapidly and the trunk undulations, which are rapid and of small amplitude, cause the body to quiver. The resident male tends to keep his body between the center of the territory and the intruder, adjusting his position accordingly if the intruder turns to the left or right; this functions to herd the intruder out of the territory. Like chasing, escorting is the functional antithesis of driving in that, in driving, the object is to force the female to the

center of the territory.

The impression given was that the escorting male was in an ambivalent state between the tendency to exhibit lateral display and the tendency to give chase--escorting may be a compromise between the two. It is significant that escorting was directed mainly toward large intruders (Fig. 6). A smaller intruder (= low threat?) usually elicited an abrupt chase from the resident while territorial males (= high threat?) elicited lateral display or some other, more restrained response. Escorting often occurred when the male was confronted with a large (= high threat?) non-aggressive (= low threat?) male.

Reciprocal pendulum Movements*. This term was taken from Tinbergen (1952). The behavior occurs between territorial males and sometimes between two fish defending feeding territories. It consists of two fish chasing one another back and forth across the boundary between the two territories. As they near the center of the territory of the one being chased it turns and chases the other toward the center of its respective territory where the reverse may occur. Sometimes this does not include full chase or flight, but only intention movements; in these instances each fish pivots back and forth around its vertical axis, first turning toward the opponent as if to chase and then facing away as if to flee.

Fleeing. The fish swims away from the attacking fish. This occurs at graded intensities depending apparently on the fright level in the fleeing fish. At highest levels the fish usually darts evasively toward the surface, or into the cover of vegetation. As described above, large males sometimes move slowly away from the challenging male, apparently

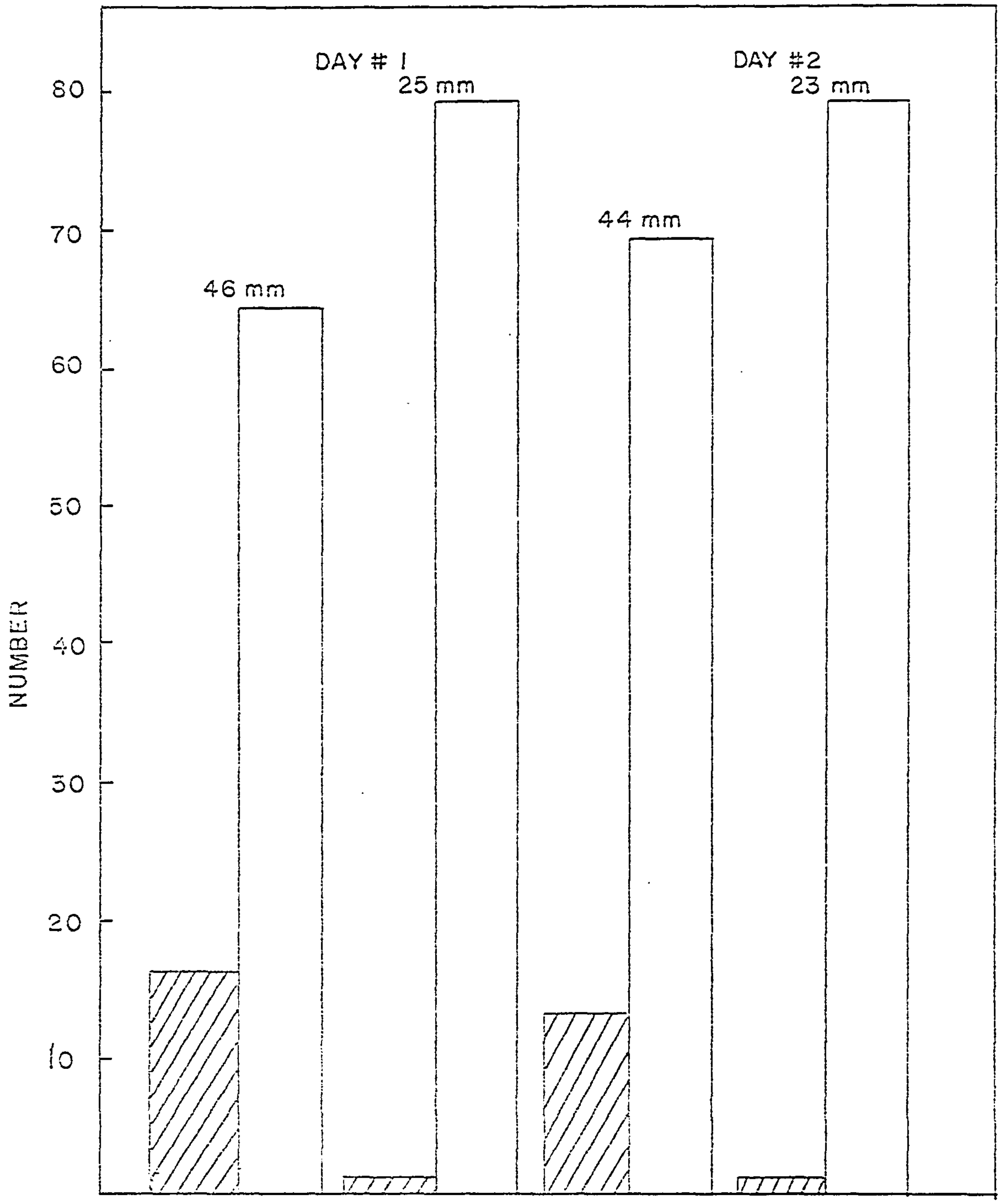

Figure 6. Relationship between the size of an intruding male and the frequencies at which escorting and chasing are exhibited by territorial males. Cross-hatched bars = escorting; open bars = chasing; total-lengths of the intruding males are given above the bars. These data were obtained by removing a selected male from a large holding tank and placing it in a 5-gal. aquarium with a stable social hierarchy. After 30 min., counts were made of the first 10 agonistic responses of two chosen territorial males to the transplanted male. This was done using 4 pairs of territorial males in four separate aquaria, for a total of 80 counts per transplanted male.

because of relatively low fright levels.

*T-circling**. This has not been described for any species of *Cyprinodon*. It was seen twice in this study in aquarium fish. On both occasions a female was attempting to feed on the bottom within a male's territory. She nipped repeatedly at the substrate keeping her tail directed toward the side of the resident male as he moved in an arc to the left and right behind her in what appeared to be thwarted attempts to either move along-side as in courtship or chase her away--finally the female fled from the territory. Mertz and Barlow (1966) and Kaill (1967) described T-circling as a courtship behavior in *Jordanella* and Kaill (*l.c.*) described it as agonistic behavior in male-male encounters in *Floridichthys*.

Tail-beating. The fish tilts head-up at about a 45-degree angle with the body bent in a C-shape, concave side toward the opponent; then it delivers one or two heavy beats of the caudal fin toward the opponent. This may be common among cyprinodontids. Barlow (1961) described it for *C. macularius*; Foster (1967) observed it in *Aplocheilus lineatus* (subfamily Rivulinae) and *Aphanius cypris* (subfamily Cyprinodontinae); and I have seen it in *C. rubrofluviatilis* and *C. elegans*.

Relative to the time spent watching the two species in aquaria, tail-beating occurred much more frequently in *C. elegans* than in *rubrofluviatilis*. It was uncommon in the latter and frequently seen in the few *elegans* that were obtained.

Appeasement posture. The fish rests in mid-water at about a 30-degree angle, tail often resting on the substrate. This was often observed in aquarium fish that had been heavily beleaguered by territorial males.

Burying. The fish tilts and "swims" into the sand. The initial angle is about 60-90° from the horizontal. As the fish penetrates deeper the body becomes more horizontal until, toward the end of burying, the head is canted slightly upward so that the fish is uncovered to varying degrees. This occurs very rapidly, requiring less than a second in loose sand. When buried, the caudal fin is usually bent to the left or right and the trunk may be bent into a sigmoid flexure. These can be considered "intention-swim" postures (Barlow, l.c.) and presumably facilitate quick-starting should the fish be stimulated to flee.

Burying might be an effective fright response against piscivorous birds (belted kingfishers, Megaceryle alcyon, and great blue herons, Ardea herodias, were seen near certain study sites) and other predators (e.g., raccoons, Procyon loter), perhaps even other fishes, but it is ineffective against conspecifics. It was never seen as a response to an attacking pupfish. On one occasion a territorial male allowed a buried pupfish to remain near the center of his territory for several minutes. He apparently was aware of the interloper's presence since he stopped nearby several times, hovered, and tilted down in an investigative manner before finally evicting it. Territorial males nip at exposed portions of the body in dislodging a buried pupfish--almost invariably causing it to flee. Burying has been described for C. macularius (Cox, 1966), C. variegatus (Raney, et. al., 1953), an unidentified Cyprinodon (Minckley and Itskowitz, 1967), Lucania parva (Hubbs and Miller, 1965), *Fundulus zebrinus* and *F. heteroclitus* (see Minckley and Klaassen, 1969).

Maintenance Motor Patterns

Chafing. Kaill (1967) noted for *C. variegatus* that "...the central objective [of this motor pattern] seems to be to scratch." Chafing is done on the bottom substrate or on particular objects; e.g., twigs or stones. It is often very erratic, especially if the chafing material is the substrate; in these instances the fish "skims" rapidly over the surface, scraping one side and then the other against the (presumed) irritated part on the object and then makes a forceful forward and upward movement, scraping the part on the object. Chafing is commonly seen in a variety of fishes (pers. obs.).

*Fin flickering**. While hovering or moving slowly forward, one pectoral fin begins to flicker rapidly back and forth, out of phase with the other pectoral. Chafing and fin flickering sometimes occur one after the other. In one instance a fish chafed a pectoral fin on a stone, and then three bouts of fin flickering immediately followed in the same fin. This suggests that, like chafing, fin flickering may be a response to irritation. A common irritant may be the copepod, *Lernea*. A number of pupfish were found with one or two of these parasites in the axila of the pectoral fins.

Yawning. The mouth opens maximally with the premaxillary extended and held momentarily. Kaill (1967) stated for *C. variegatus* that, in yawning the "...fins are raised and the body is thrown into a sharp sigmoid curve with the caudal fin abruptly brought into a position about 45° from horizontal." This was not noted in _C_. _rubrofluviatilis_. Fin raising is commonly associated with yawning in centrarchids (Miller, 1963; pers. obs.).

_S-shape and anal fin wrap_*. The body is thrown into a sigmoid flexure and the anal fin is bent to one side and curved upward at the tip to form a "cup"--exactly as seen in wrapping during spawning. This is sometimes followed by a sharp turn in the direction that the head is turned. This was noticed only in males; thus it may represent a behavior _in vacuo_, i.e., it may be the same as wrapping in reproductive context, but without the usual stimulus. However, it was not established for certain that this does not also occur in females. Hemens (1966) described a similar comfort movement for _Xiphophorus hellerii_ in which it involved gonopodial swinging, and a similar behavior has been described by other workers for _Lebistes_ (see Hemens, _l.c._). Hemens (_l.c._) mentioned that the copulatory movement in those forms with gonopodia may be an evolutionary derivative of this comfort movement. A similar hypothesis could be proposed for _C_. _rubrofluviatilis_--an interesting bit of conjecture is that anal fin wrap in the Cyprinodontidae and gonopodial swinging in the Poeciliidae are homologous behaviors with a common ancestry.

Resting and Sleeping. The fish usually rest on the bottom, either on the surface of the substrate or else buried with only the head exposed. When sleeping they usually rest on the surface in an upright posture supported on both sides by the pectoral fins. Burying was most commonly observed in nature during cold weather, but even then most of the fish rested on the surface of the substrate. Cox (1966) noted for C. macularius in Arizona that beginning in October the adults burrowed into the mud and debris at the bottom of the pond where they remained dormant until April.

Territoriality in Red River Pupfish

Following Noble's (1939) interpretation, a territory is herein considered as being any defended area. Territorial behavior is defined as behavior that serves to establish and maintain a territory (Assem, 1967). Functionally, there are two distinct categories of territoriality in Red River pupfish, one seeming to enhance the breeding efficiency of the territorial resident, while the other seems to enhance feeding efficiency. These are referred to below as "breeding territoriality" and "feeding territoriality", respectively. The latter has not been described for any Cyprinodon.

Physical aspects of breeding territories. Territorial behavior in breeding context was confined to particular areas that usually were well defined topographically. Most of the territories were situated in communal breeding areas that supported a number of adjoining breeding territories. These areas were in shallow water to depths as great as 50 cm in the following general situations: (1) flat shoals where the current was minimal; (2) around the margins of pools in areas of both flowing and intermittent waters; (3) small coves isolated to varying degrees from the current; and (4) in quiet water over large prominences such as boulders and piles of woody debris.

Territories ranged in size from diameters of two or three times the length of the fish to as large as 4 m^2 in area. They tended to be larger in flat open areas than in areas where the surface of the substrate was broken with vegetation, stones, debris, etc. In several instances, a number of males were defending small territories over heaps of woody debris on the stream bottom. In areas where territories were dense, larger males defended larger territories in more favorable locations than smaller males. Small males (less than 20 mm, TL) were frequently seen defending sub-territories between the territories of larger males and sometimes in areas at the water's edge too shallow for the latter to enter. In aquaria, they often were confined to small territories in upper corners of the tanks. Territories were smaller in areas with large numbers of territorial males than where they were fewer in number.

The territories were variable in shape, but tended to be roughly circular in flat open areas. In areas with slight to moderate current, they were elongated in the direction of the current. The shape was partly dependent upon the behavior of surrounding males, tending to bulge toward less aggressive neighbors and to recede from more aggressive ones. One male's territory consisted of a loose "brushpile" to which he was restricted by the aggression of neighboring males; accordingly, the outline of his area was very irregular as it closely corresponded to the limits of the brushpile.

Aquarium observations showed that the territories have a roughly "dome-shaped" three-dimensional aspect, with a maximum height of approximately 10 cm at the center sloping downward on all sides toward neighboring territories. Intruders from above could descend farther to the bottom without interference from territorial males in zones over the boundary between territories. This is apparently a corollary of Tinbergen's (1951) description of how aggression is dependent upon how far the resident is from the territorial center, with maximum aggression exhibited nearer the center.

Aggression associated with breeding territoriality. In late spring and summer the breeding males tended to defend their territories continuously during daylight hours, even when spawning had ceased for the day. In the winter, territorial defense began when the water temperature neared 55 F and continued until nightfall. In territorial defense the male patrolled back and forth at moderate to rapid speeds, pausing occasionally to nip or dig and often posting for brief periods. Frequent stops were made at the territorial boundary, usually in response to the close proximity of a neighboring male. Most of the challenges and fights between neighboring males occurred at the boundaries between territories. Patrolling tends to carry agonistic encounters with neighbors to the periphery of the territory; thus maintaining the integrity of and reinforcing the territorial boundaries.

The resident males chased all intruders, juveniles as well as adult males and females, from the territory, usually with a simple charge or chase. It was sometimes difficult to determine whether a female was being chased from the territory or courted. In courting, the male made sweeping movements that tended to drive her toward the center of the territory while in chasing he tended to do the opposite. The frequency of these male-female encounters was different depending upon whether reproduction was occurring. During the reproductive period of the day the males tended to court every female, while at other times they almost always chased them. Larger males sometimes elicited escorting in the resident male.

Encounters between neighboring territorial males often consisted simply of one facing away from the approach or challenge of another and moving toward the center of its own territory. More prolonged encounters were common, especially at times of intense reproductive activity and when territories were being established in aquaria and in nature. In the summer there was a period of intense aggression just before sunrise when spawning activity was sporadic, possibly representing reinforcement of territorial boundaries after the night-time period of relative inactivity. Longer encounters occurred primarily at the boundaries between territorial males; they sometimes consisted of mutual facing, and then a shift to eyeing or arching.

During eyeing or arching, one male often faced away and moved off, leaving the other in place, or else the second followed in pursuit. After facing away, the first often turned and eyed or arched again, particularly if pursued, whereupon the other returned the challenge or moved toward the center of his own territory; this occasionally evolved into reciprocal pendulum movements. More rarely, one male tilted up during arching, delivered one or two tail-beats at the other and then moved away.

After arching, the two combatants sometimes began circling, a development that frequently evolved into mouthfighting. Circling and mouthfighting occurred primarily between males that were near equals in size and/or aggressiveness. These bouts usually lasted a matter of several seconds at the most, and often ended with mutual challenge, either eyeing or arching, after which the encounter was terminated as one or both combatants moved away--or else fighting began anew.

Courtship and spawning. Late in the reproductive season, in October and November, the males frequently courted females over the general area with little regard for territorial boundaries; this was a rare occurrence earlier in the reproductive season when spawning was at its peak of intensity. Spawning activity of individual males was almost invariably confined within the boundaries of their respective territories. Since the males are relatively stationary, a prerequisite for spawning is that females approach the territories.

Females commonly wandered individually into the territories of reproductive males while foraging, and schooling females frequently passed through the territories of one male after another in moving between feeding areas, in moving from night-time resting areas to feeding sites, and when participating in the upstream migration that occurred in the Childress area. These male-female encounters usually seemed incidental to the movement of females from one place to the next, i.e., females did not appear to be attracted to the males for reproductive functions. Such females frequently fled from courtship with one male into the territory of another which also courted her. In this manner a female sometimes spawned with several males in succession. On a few occasions, individual females swam directly toward the center of a territory as if to attract the male; spawning usually required little preliminary courtship in those instances.

At peaks of reproductive activity small clusters of females were common in neutral areas between territories where they were relatively free of aggression from territorial males. However, males made frequent sweeps into them, often succeeding in separating one from the group and driving her toward the territorial center where courtship and, less frequently, spawning ensued. The success of the males in separating females from these clusters was dependent upon fright responses of the individual females. The females made short evasive movements in all directions as the males swept through their midst; then the males usually doubled back and courted any female that happened to flee into their respective territories. Resident males behaved similarly toward schools moving nearby or through their territories.

Figure 7 shows an idealized courtship sequence for Red River pupfish. If the female was swimming toward the territorial boundary the male exhibited zigzagging or herding, depending upon whether she was in mid-water or near the bottom, respectively. Flight to the surface was an effective method used by pupfish in avoiding the charge of an aggressive male, and it was commonly exhibited by females that were fleeing in response to the male's courtship antics. Zigzagging seems to function to inhibit forward movement and to lead the female to the substrate,

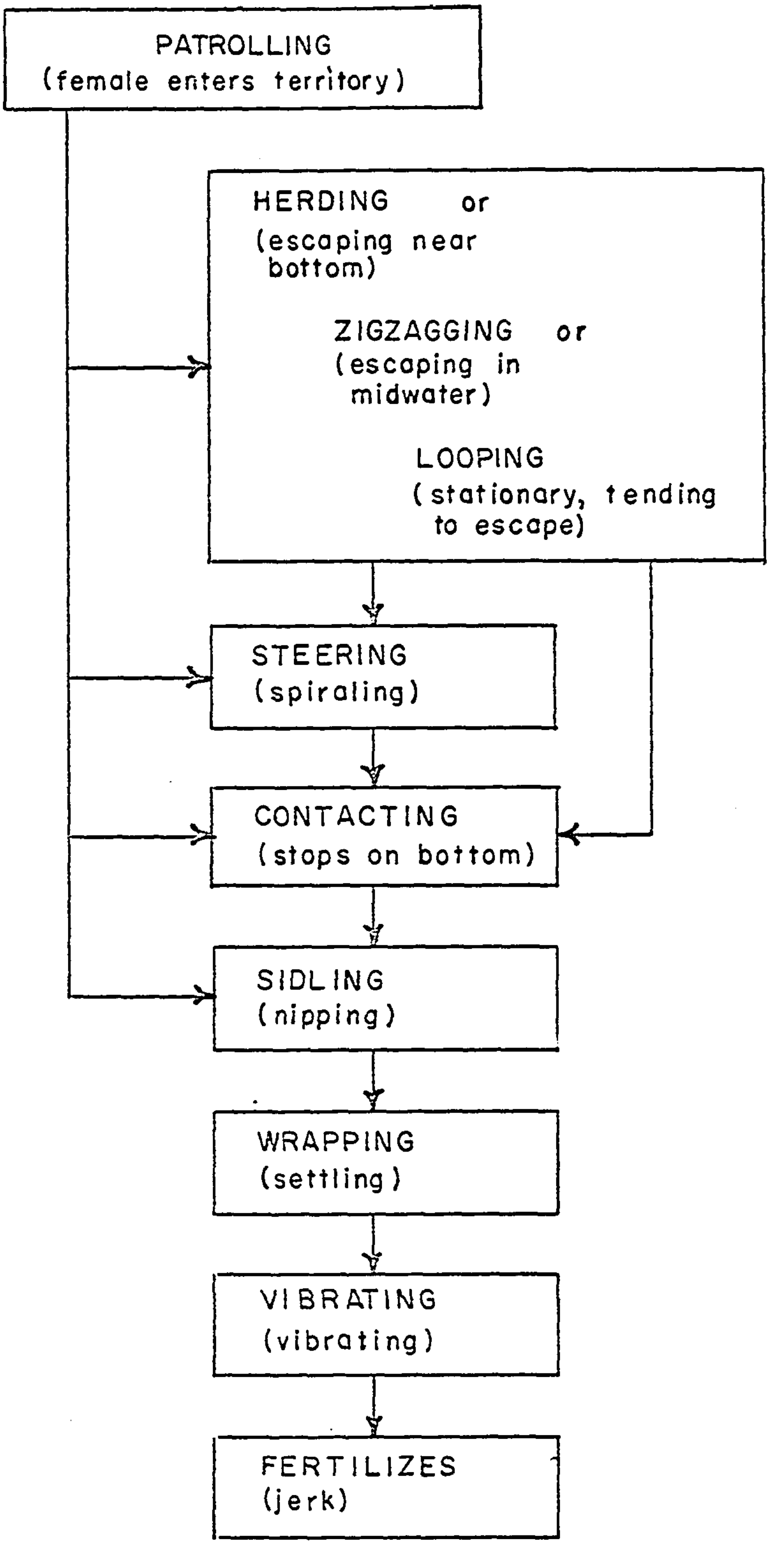

Figure 7. Idealized courtship sequence in Red River pupfish. Capitalized names = male behavior; parentheses = female behavior.

while herding functions to turn her away from the territorial boundary. Looping was elicited when the female was relatively stationary in midwater or on the bottom. The stimulus for looping seems associated with a tendency for the female to flee; it may function to inhibit her forward movement and, if she is in midwater, to lead her to the bottom.

In more direct courtship sequences, the female swam deep into the territory near the bottom and remained relatively stationary as the male rushed to her and moved alongside; courtship in these instances usually began with steering, contacting, or sidling. The males are very adaptable, being able to vary the courtship sequence in a number of ways depending on the behavior of the female (Figs. 8 and 9). If she attempted to escape at any stage of courtship, including the consummatory phase, the male usually reverted to driving, looping or zigzagging in efforts that seemed aimed at keeping her within the territorial boundary; sometimes the female fled from the territory with the male making no attempts at further courtship.

The relative frequencies of driving, looping, and zigzagging were variable depending upon the time of year at which observations were made. In the summer herding and steering were the predominant preliminary stages of courtship while looping and zigzagging were rarely seen (Fig. 8). Toward the end of the reproductive season looping and zigzagging were seen much more frequently, and steering was practically absent (Fig. 9). Although herding occurred at a relatively high frequency during both periods, it was very brief late in the season and usually consisted of little more than the male moving so as to place himself in front of the

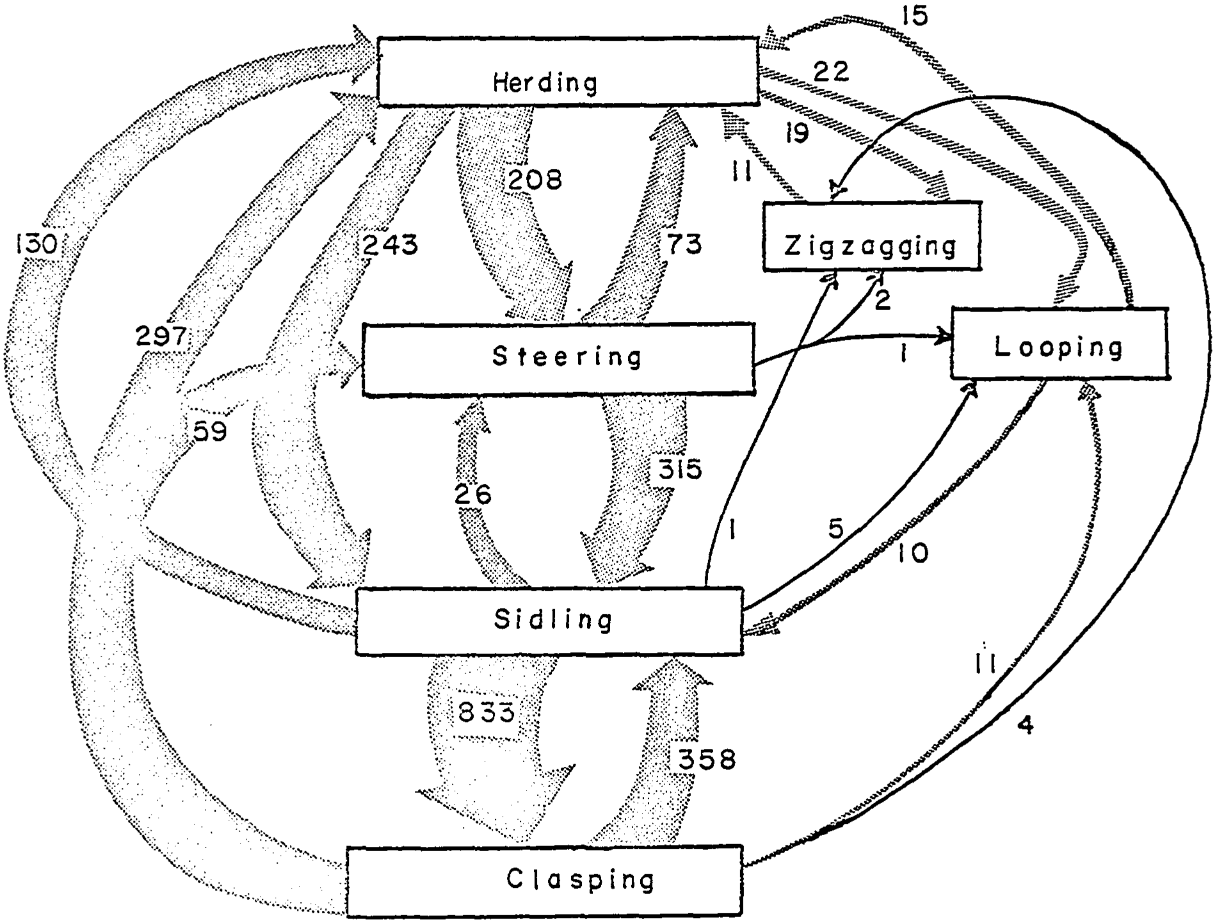

Figure 8. Courtship activities of male Red River pupfish on July 30, 31, and August 1, 1969. Taken from data on the activity of approximately 25 males in a 25-foot segment of Oscar Creek. Superimposed numbers = the number of times the transitions between activities were seen. Only those sequences of more than two activities were considered.

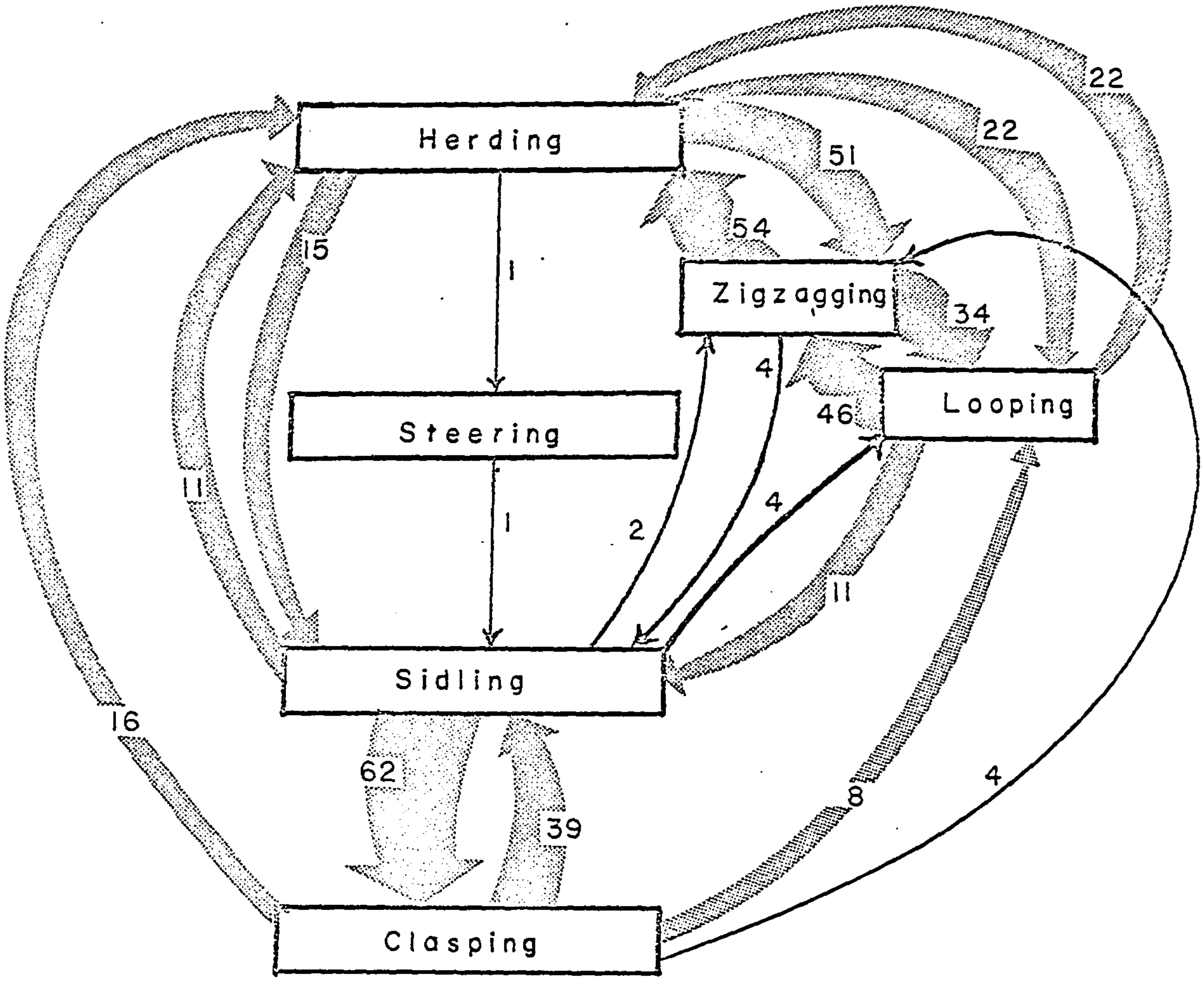

Figure 9. Courtship activities of male Red River on October 27 and November 1, 1969. Taken from data on the activity of approximately 10 to 15 males on both dates in the same 30-foot segment of Oscar Creek. Superimposed numbers = the number of times the transitions between activities were seen. Only those sequences of more than two activities were considered.

female where zigzagging or looping ensued. Prolonged herding was common in the summer, but it was rarely seen later in the reproductive season--instead, looping and zigzagging were the prolonged phases of courtship.

The seasonal shift in courtship activities may have been the result of a change in female receptivity, with their being more receptive in the summer than in the fall. Evidence for this conclusion is that, of 707 courtship encounters represented by Figure 8, 70% (495) led to clasping while of the 158 encounters represented by Figure 9, only 4% (13) led to clasping.

Courtship usually ended with the female fleeing as the male attempted to contain her within his territory. This is exemplified by the large number of encounters ended with the male herding, steering, zigzagging, and looping (Fig. 10). A large number of encounters were terminated with clasping; this was expected, since the consummatory act (spawning) occurs in this phase. Not all encounters ended after spawning, for the pair often spawned several times in succession before separating. Excepting those terminated due to interference from intruding males, most encounters were terminated by evasive movements of the female; however, a few ended with the male deserting the female for no apparent reason. In several cases he deserted after courtship had proceeded to vibrating--on one of these occasions the female continued vibrating in his absence for a second or so before swimming away.

Male-male interactions associated with courtship and spawning. Aggression between territorial males was maximal at times of intense

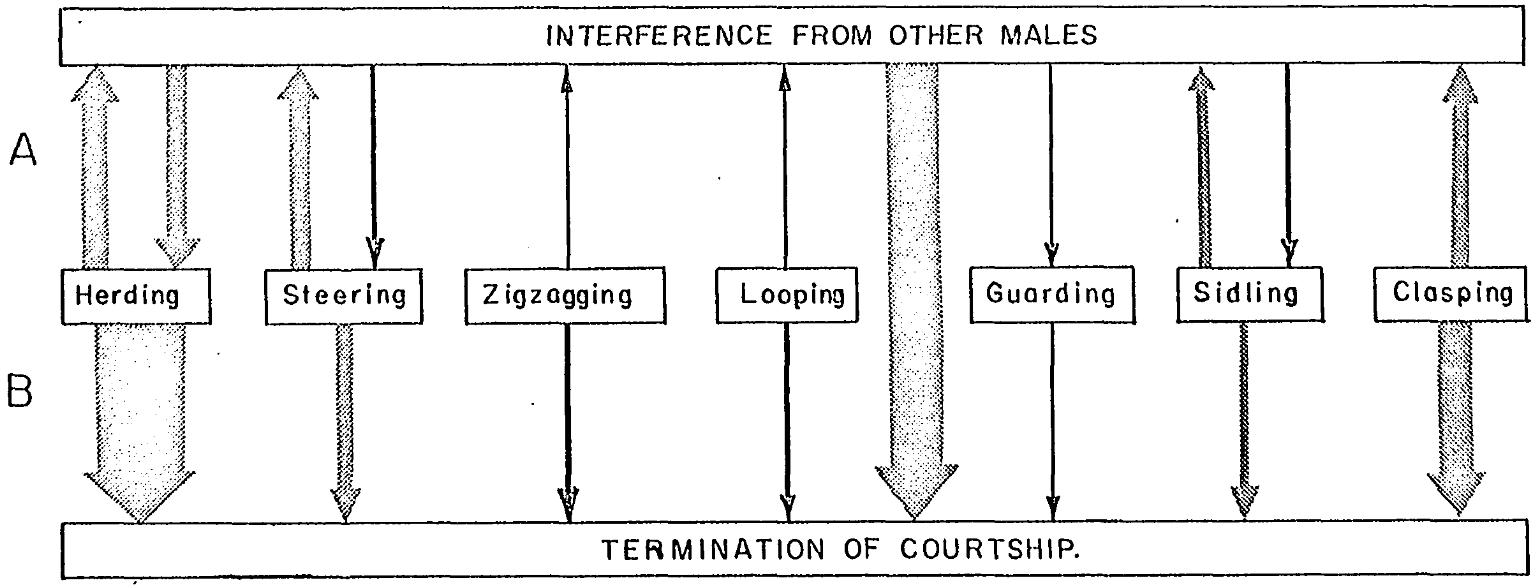

Figure 10. Relationship between the termination of male courtship activities and interferences from other males. (A) ↑ = the phase of courtship at which interfering males intruded; ↓ = the phase initiated in those instances when courtship was resumed after the intrusion. (B) the phase at which courtship was terminated with female flight. Widths of the arrows are proportional to the frequencies of occurrence. This figure is based on the courtship sequences represented by Figure 8.

spawning, e.g., from dawn until around noon in the summer (Fig. 4). Aggression was less likely to be confined to territorial boundaries at these times, for males sometimes moved into neighboring territories in competition for receptive females. Most of this type of aggression occurred when a male was courting near his territorial boundary. This commonly attracted neighboring males and often led to fighting.

Interferences from other males were most frequent in early courtship when the courting male was driving (= herding and steering) the female toward the center of his territory (Fig. 10). Herding was often prolonged, and as the pair chased about the territory they usually approached the territories of neighboring males. This sometimes caused a male to charge or challenge the courting male directly or to make sweeps at the female in apparent attempts to herd her toward his own territory; this sometimes occurred with several males in succession. Such activity often resulted in aborted courtship as the female fled, frequently into the territory of the intruding male.

Interference also occurred in later stages of courtship, i.e., steering, sidling, and clasping. The intruder sometimes charged boldly toward the pair and physically disrupted their activity. Infrequently, neighboring territorial males rammed into the side of the male of a clasping pair. This usually disrupted the spawning act causing the female to flee from the area; again, this was most likely to occur when the spawning pair was located near the boundary of a neighboring territory. Interfering males usually approached hesitantly and paused a few centimeters away; this frequently caused courting males to desert the female to challenge or chase the intruder away. An occasional male tried to force himself between the clasping pair from behind; they were always chased away by the spawning male. Small males sometimes S-shaped and vibrated with a spawning pair by sidling against the female from the side opposite the other male. Functionally, these activities are the same as "sneaking" in Gasterosteus aculeatus (see Assem,1967) in that males unable to establish territories due to the aggression of others may occasionally fertilize eggs that are deposited by females in response to the courtship activity of territorial males.

The courting male sometimes returned to the female and resumed courtship after an interruption by another male, but more often than not, courtship was terminated by the interference (Fig. 10). From this it can be concluded that interferences with neighboring males are a significant factor in determining the reproductive success of individual males.

Routine aggression and feeding territoriality. Aggression in the general pupfish population, excluding that related to breeding activity, usually occurred between members of feeding aggregations. Very little aggression occurred during periods of inactivity, as when most of the population was resting on the bottom at night or in response to low temperatures. Pupfish were non-aggressive when schooling, but aggression intensified as soon as the school stopped moving and the individuals began feeding. Aggressive activity at these times consisted primarily of one individual being displaced or supplanted by another and of avoidance behavior where fish steered away from others or caused them to move out of their paths of movement. This was similar to that described for the dogfish, Mustelis canis, by Allee and Dickinson (1954). Smaller pupfish tended to avoid larger fish more so than vice versa and silver-iris fish seemed more liable to be displaced than black-iris fish (see "color patterns" section).

More intense aggression occurred when members of the population began defending feeding territories. Feeding territories were small circular areas only slightly larger than the length of the fish. They were sometimes fairly fixed in position while at other times they were more mobile, with the fish briefly defending one spot against intruders then moving and defending another spot.

Fish defending feeding territories invariably had a black iris, and tended to feed intensively in one spot. Silver-iris fish seemed to wander more over the general area, feeding as they moved about. In territorial defense, the resident remained largely in one spot pivoting to the left and right investigatively and continuously warding off approaching fish, usually by turning and directing aggressive intention movements or thrusts toward them. Low intensity arching and,less frequently, tail-beating were sometimes seen in disputes over these territories. Plowing sites always represented feeding territories. As soon as a fish began plowing, it immediately became aggressive toward all other fish, chasing them away as they neared the site. Feeding and plowing were attractive to conspecifics and where the population was dense the resident was frequently confronted with intruders. The resident usually defended successfully against smaller or equally sized fish, but its position was sometimes usurped by larger pupfish. At plowing sites, the latter often began feeding and plowing and chasing others away.

At temperatures above 90 F territorial males often deserted their breeding territories and migrated to deeper water where they usually defended small feeding territories that were relatively stationary. Others defended small areas within the larger breeding territory, and still others, especially those in deeper water, continued defending breeding territories--even when reproduction had ceased for the day. The initiation of feeding territoriality in breeding males was roughly coincident with increased feeding activity in the general population, including the males that continued defending breeding territories. These observations suggest that the motivational basis of "feeding" territoriality is indeed related to the feeding drive. Further evidence for this conclusion is the observation that the resident fish fed very actively in its territory, plowing and/or nipping and digging continuously when not warding off other pupfish.

The tendency for males defending breeding territories to defend feeding territories at high temperatures may partly be the result of a lowering of the metabolic "scope for activity" (see Fry, 1957) by the higher temperatures. With increasing temperature the metabolism required for body maintenance may increase to the point where there is no energy for the sustained periods of patrolling, chasing, and generally high level of activity required of males defending breeding territories. Then they either migrate to cooler waters, or restrict their activity to defense of smaller feeding territories. Furthermore, they can feed more actively in the feeding territory than when defending breeding territories; thus meeting the demands for increased nourishment at higher temperatures.

Aggressiveness in feeding may serve to insure adequate feeding space for individuals. Herein probably lies the adaptive significance of aggressiveness in the feeding context--an aggressive pupfish that keeps competitors at a distance may forage more efficiently than one which feeds in closer proximity of competitors. Perhaps related to this is the development of visual intimidation signals such as the black iris. These signals could operate at a distance without the necessity of overt aggression, and in this way they may partially detract from the increased energy expenditure associated with higher levels of aggressiveness and social interaction.

Functions and evolutionary implications of breeding territoriality. A number of possible functions could be listed for breeding territoriality in the Red River pupfish, but the following seem most probable: (1) protection of the fertilized eggs and (2) provision of sufficient space to allow the male to carry out courtship maneuvers in relative freedom from interference by conspecific males.

I have, on two occasions, seen pupfish in aquaria feed on recently spawned eggs. The eggs are readily susceptible to predation by bottom-feeding fishes, especially the pupfish since they are intense bottom-feeders. This effect is heightened by the fact that they usually feed in large aggregations. Such aggregations could rapidly decimate the egg "population" in an area were it not for the territoriality of the breeding males. Perhaps related to this is the observation that resident males also evict other species of fishes from the territories. In aquaria, conspecifics were sometimes allowed to remain in a territory for variable lengths of time as long as they remained inactive; however, as soon as one tilted to nip at the bottom, it was abruptly evicted by the resident male. This function of territoriality is similar to that performed by fishes that defend "nests" in which the eggs are deposited.

An evolutionary concomitant of the egg-protecting function may be the apparent tendency for males defending breeding territories to feed less than females (Fig. 5). Barlow (1964) stated: "Most likely the first kind of parental behavior [in fishes] other than preparing the site was the tendency to remain with the eggs without eating them." It may be that, within the cyprinodontines, territorial behavior as exhibited by Cyprinodon adumbrated the somewhat more specialized parental behavior described by Mertz and Barlow (1966) for Jordanella.

The second function listed above for breeding territoriality follows from the observation that interferences from other males frequently led to aborted courtship (Fig. 10). It is easily seen that aborted courtships would be much more frequent were it not for territoriality. By establishing a territory the male obtains space from which other males are largely excluded, thereby diminishing the chances of interference during courtship. The probability of interference is further lowered if the territory is fairly large, for the chances that a courting pair will encounter a neighboring male are fewer in larger territories. A major factor limiting the size of the territory is probably the amount of area that can be successfully defended against intruders. In areas where pupfish were abundant, the males were constantly on the move chasing intruders from their territories.

It is interesting to speculate on what might have been the evolutionary prototype of breeding territoriality in pupfish The nature of breeding territoriality in Red River pupfish is similar to territoriality as described for other Cyprinodon (Barlow, 1961; Cox, 1966; Kaill, 1967; Foster, 1967; Raney et al, 1953), and it is similar to Jordanella in that the defended area is topographically fixed and almost perpetually kept clear of intruders (Mertz and Barlow, 1966); Kaill (l.c.) observed that Floridichthys males defend shifting territories that are "without geographical reference"; Foster (l.c.) stated that the Syrian cyprinodontine, Aphanius cypris, defends its territory almost continuously, regardless of the presence of females; and regarding the subfamily Fundulinae, Foster (l.c.) stated that in most species the "...territories are only defended for a portion of the day, and the most vigorous agonistic encounters between males only occur when a receptive female has entered the territory of one of the males." Considering the Cyprinodontinae and Fundulinae as a whole, it appears that the most universal function of territoriality is to enhance the reproductive success of the individual male by keeping competitors at a distance. This function would be served in all species, including those with ephemeral territories. Hypothetically, territoriality in the ancestral Cyprinodon might have existed only with reference to the female being courted. This "primitive" territoriality might have been similar to behavior exhibited in the instances in which male Red River pupfish courted females without regard to territorial boundaries. In these instances the courting male behaved aggressively toward other males and tended to station himself between them and the female.

Perhaps in response to derived agonistic advantages such as the "prior residence" effect (see Braddock, 1949) the territory then became geographically fixed. Following this, courtship behavior (e.g., driving) aimed at keeping the female within the confines of the territory might have developed as modifications of more basic motor patterns (e.g., looping and zigzagging) aimed at slowing her progress to allow spawning to ensue. Finally, the tendency to defend the territory continuously during daylight hours, regardless of whether or not reproduction was occurring, could have occurred in response to the selective advantage of affording protection for the fertilized eggs from the feeding population.

Evolutionary concomitants of territoriality in breeding males probably were the development of (1) conspicuous color patterns and (2) a large body surface for the display of these patterns. Related to this is the observation (Miller, 1948) that the male Cyprinodon is usually deeper-bodied than the female. Conspicuousness might advertise the male's presence, thereby attracting sexually motivated females and warding off breeding males and members of the feeding population at the same time. This effect is probably enhanced by lateral display within the territory.

Lateral display in male pupfish involves the spreading of all fins, including the pectorals and pelvics which are rotated so that they extend below the ventral line of the profile; similar behavior is common in fishes. An obvious result of lateral display is that the largest aspect is presented to antagonists. Related to this is the oft-noted observation that size is important in determining social status, with larger fish tending to have the advantage (e.g., Allee _et al_, 1948; Braddock and Braddock, 1955; Caldwell and Caldwell, 1962; Miller, 1964; and Newman, 1956). Apparently it is a general rule among fishes that large size tends to inhibit attack. Indeed, it seems as if territorial pupfish males deal more cautiously with larger intruding males than with smaller intruders; the latter elicit an abrupt chase from resident males, while large males often elicit escorting (Fig. 6). Barlow (1961) stated that in _C_. _macularius_, "the size of the opponent appears to determine the first response of a territorial male." Accordingly, it seems that, within limits, there would be adaptive premium for any factor that would allow the presentation of a large image in aggressive encounters.

As previously noted, the male Red River pupfish is disproportionately deeper-bodied than the female. An additional value of this phenomenon, not directly related to the display of breeding coloration for increased conspicuousness, might be the increased size of the aggressive image presented to antagonists, an effect that would inhibit physical attack from rivals. The deep-bodied aspect of male _Cyprinodon_ may be an evolutionary parallel of lateral display aimed at increasing the size of the aggressive image. Assuming that there is selective value in presenting a large image, then factors such as lateral display and increased body depth would have obvious adaptive value. Similarly, chromatic factors that emphasize the effects of such behavioral and structural adaptations would also have adaptive value (see "color patterns" section).

It is tempting to think of breeding territoriality as being an elaboration of feeding territoriality. Functionally, they seem basically the same: (1) competitors for food on the one hand and females on the other are kept at a distance, and (2) food sources, general food items on the one hand and eggs on the other, within the territories are protected from the general feeding population (even though the "protected" source is eaten by the resident in feeding territories).

Behaviorally, the major differences between feeding territoriality and breeding territoriality are: (1) breeding territories are more permanent, (2) patrolling, a behavior that functions to carry agonistic encounters farther from the center of the territory, is absent in the feeding context, and (3) reproductive behaviors occur in breeding territories and not in feeding territories. The motor patterns involved in interactions with conspecifics are basically the same, regardless of whether the resident is defending a feeding or a breeding territory--the major differences being that in the latter they are somewhat more ritualized and occur with higher intensity. As an example, circling in defense of a breeding territory has its counterpart in feeding context. The motivation behind circling in breeding context seems to be the tendency to remain in place in spite of the opponent's aggression. Similarly, residents of feeding territories frequently move in a tight circle in response to the approach of a large fish, or a fish from behind--circling brings it back to its feeding site and at the same time allows it to avoid the aggressor. The resident often displaced the intruder by approaching it from the flank at the end of the maneuver. Circling in breeding context is more repetitious and faster than that just described, perhaps as a reflection of the higher aggressive level in breeding males.

Patrolling and posting also have their counterpart in feeding territoriality. The constant rotation about the vertical axis usually seen in residents of feeding territories are similar in appearance to posting, and both types of behavior seem to be investigative in function. The development of patrolling would simply involve locomotion to the territorial boundary after a pivot in a particular direction.

Breeding territoriality in Cyprinodon may consist of the basic components of feeding territoriality (e.g., the tendency to orient aggression around a particular spot on the bottom) superimposed upon the "primitive" type where the territory existed only with reference to the female being courted. Patrolling might have evolved to increase the size of the territory for protection against interfering males during courtship procedures.

CHAPTER V

COLOR PATTERNS AND THEIR SIGNIFICANCE

Introduction

Barlow (1963) stated that, "Color changes [in fishes], or the existing color pattern, frequently indicate motivational states in the absence of other overt behavior, or they may constitute an additional source of information for constructing an [ethological] argument." He pointed to the paucity of papers dealing in detail with the motivational bases and signal value of color changes in fishes.

Few papers have examined the causative factors in moment-to-moment changes in color pattern. Notable exceptions are papers by Baerends _et_ _al_ (1955) and Barlow (1963) on the guppy, _Lebistes_ _reticulatus_, and the Asian teleost, _Badis_ _badis_, respectively. Neil (1964) presented a quantitative analysis of color changes in _Tilapia_ _mossambica_.

Studies concerned with coloration in cyprinodontids have dealt primarily with the physiology (Waring, 1963, gives a review of studies on _Fundulus_) or with general descriptions of coloration (e.g., Foster, 1967; Kaill, 1967; Newman, 1907; and Raney _et_ _al_, 1953) that usually emphasize sexual dichromatism. Momentary changes in coloration have been largely ignored. Barlow (1961) discussed certain chromatic elements of _C_. _macularius_, briefly considering certain related fishes

(C. variegatus, C. nevadensis, Aphyosemion, and Fundulus).

My purposes in this discussion are to (1) describe the repertory of color patterns in C. rubrofluviatilis, (2) relate the color patterns and moment-to-moment changes in pattern to the behavior of the fish, and (3) develop inferences as to the functional significance and motivational bases of various aspects of the patterns.

Component Parts of the Color Patterns

The color patterns can be broken into two major components--ground coloration and the various body markings. The markings can be resolved into seven elements: ocellus, teardrop, black iris, dorsal blotches, lateral blotches, caudal band, and vertical bars. A description of each element is given below.

Ocellus. A melanistic spot on the dorsal fin, located basally and near the posterior margin in juveniles and females; disappears in males by the time they are 25 mm in total length; usually persists in adult females, but varies from dark and distinct to barely discernible, and is absent in some; little control is exercised over the intensity, but seems to pale somewhat in extreme fright.

Teardrop. A melanistic mark beneath the eye; extends from the antero-ventral rim of the orbit to just below the level of the jaws, where it stretches anteriorly to the angle of the mouth; intensity varies from time to time, and at times the mark is absent.

Black Iris. Iris varies from silvery to black; blackening may be partial or complete, and is often confluent with the dark teardrop, sometimes making a delimitation of the two difficult. The relationship between these two marks is not clear--they usually occur concurrently, but they also occur independently of each other.

Dorsal Blotches. Reddish brown, dark brown, or olive green markings on the dorso-lateral aspect of the body, extending roughly one-third of the way down the sides; more distinct in areas posterior to the nape; seen from the side, they are square to rectangular with irregular margins; four to five may be spaced from just anterior to the dorsal fin to just anterior to the caudal origin; usually merge in passing over the caudal peduncle, but may remain separate, forming dark "saddles".

Lateral Blotches. Blotches located on the sides of the body that extend toward the venter from just below dorsal blotches. Offset and separated from dorsal blotches by an irregular lateral stripe consisting of the lighter background color of the body; number varies from four to nine; usually darker toward the upper margin and lighter toward the lower margin; sexual differences evident, even in individuals as small as 20 mm, total-length, roughly rectangular in males, while in females, they are attenuated in the lower reaches, forming a triangular mark; colors as described for dorsal blotches.

Caudal Band. Seen only in males; a vertical melanistic stripe on the posterior margin of the caudal fin; varies in width and intensity from narrow and dusky to wider and black, depending apparently on the motivational state.

Vertical Bars. Seen only in adult males; four or five vertical bars on the sides of the body that extend unbrokenly from the mid-dorsum to the venter except in the region of the nape where they usually merge to varying degrees with the colors of the dorsum; sometimes quite distinct owing to sharp contrast afforded by light background, but at other times they may be barely discernable; color varies from light grayish-blue to maroon with blue highlights; vertical bars and blotches are mutually exclusive.

Typical Color Patterns

Certain color patterns of Red River pupfish are characteristic of sustained periods of particular activities. These were given descriptive names to facilitate the discussion. The following describes and characterizes the patterns according to associated activities.

1. Blanched

The body is pale, usually silvery with golden highlights; fins pale and dusky; no conspicuous marks.

- a. Males and females in a high state of fright in aquaria.
- b. Fish "fluttering" up and down the sides of aquaria.
- c. Fish in appeasement postures in aquaria.
- d. Territorial males in the initial stages of recovery from fright in aquaria after being frightened by an outside disturbance. This is the only situation in which the black iris occurred as part of Blanched.

2. Blotched

General body coloration highly variable, may be pale or dark, and ranges from reddish to yellowish brown to various shades of brown and olive green; dorsal and lateral blotches present, fins pale, ocellus present in female; caudal band (male character) indistinct, teardrop and black iris may be present or absent (Fig. 11).

- a. Fish hiding in cover or in a darkened area of an aquarium, teardrop and black iris present or absent.
- b. Fish feeding on the bottom in nature and in aquaria, black iris and teardrop present or absent.
- c. Non-breeding males and females defending small temporary feeding sites on the bottom in nature and in aquaria. Black iris almost always present.
- d. All fish when "sleeping" at night; black iris and teardrop always present.

e. Fish participating in benthonic schools, black iris present or absent.
f. Spawning females, black iris and teardrop never present.
g. Fish resting on the bottom in the open or in cover.
h. Fish exhibiting migratory fluttering in aquaria (see Assem, 1967); black iris absent.

3. Lateral Band

General body coloration similar to that described for "Blotched" except that dorsal blotches are absent, and lateral blotches are merged to form an irregular lateral band; dorsum slightly pale; black iris and teardrop present or absent (Fig. 11).

a. Pelagic fish swimming or resting individually at the surface or at least several inches off the bottom.
b. Fish participating in pelagic schools in nature.
c. Fish feeding at the surface on floating materials.

4. Mottled

Dorsum with brown to olive green "mottling", lateral blotches absent. Coloration otherwise similar to Blotched and Lateral Band patterns.

a. Not seen as frequently as the other patterns, but sometimes seen among aquarium fish that were swimming off the bottom.

5. Blue Nape

Blue iridescence on the upper half of the body with highest intensity in the nape region; light to bright yellow in the face, pectorals, pelvics and general lower half of the body anterior to the caudal peduncle; dorsal and anal fins charcoal gray to black, with hyaline borders around the posterior edges; caudal band charcoal gray to black and variable in width being wider when the band is black and narrower when gray; black iris and teardrop always present (Fig. 11). Referred to as "high intensity Blue Nape" when colors are bright and "low intensity Blue Nape" when colors are dull.

a. Reproductive males engaged in all activities related to territorial defense and reproduction.

6. Vertical Bars

The colors are the same as high intensity Blue Nape, but with the addition of vertical bars (Fig. 11).

a. Occurrence is correlated with the same type of activities noted for Blue Nape, but as discussed below, there is a quantitative difference between activities correlated with the two patterns--Vertical Bars is seen more frequently in periods of intense reproductive or agonistic behavior.

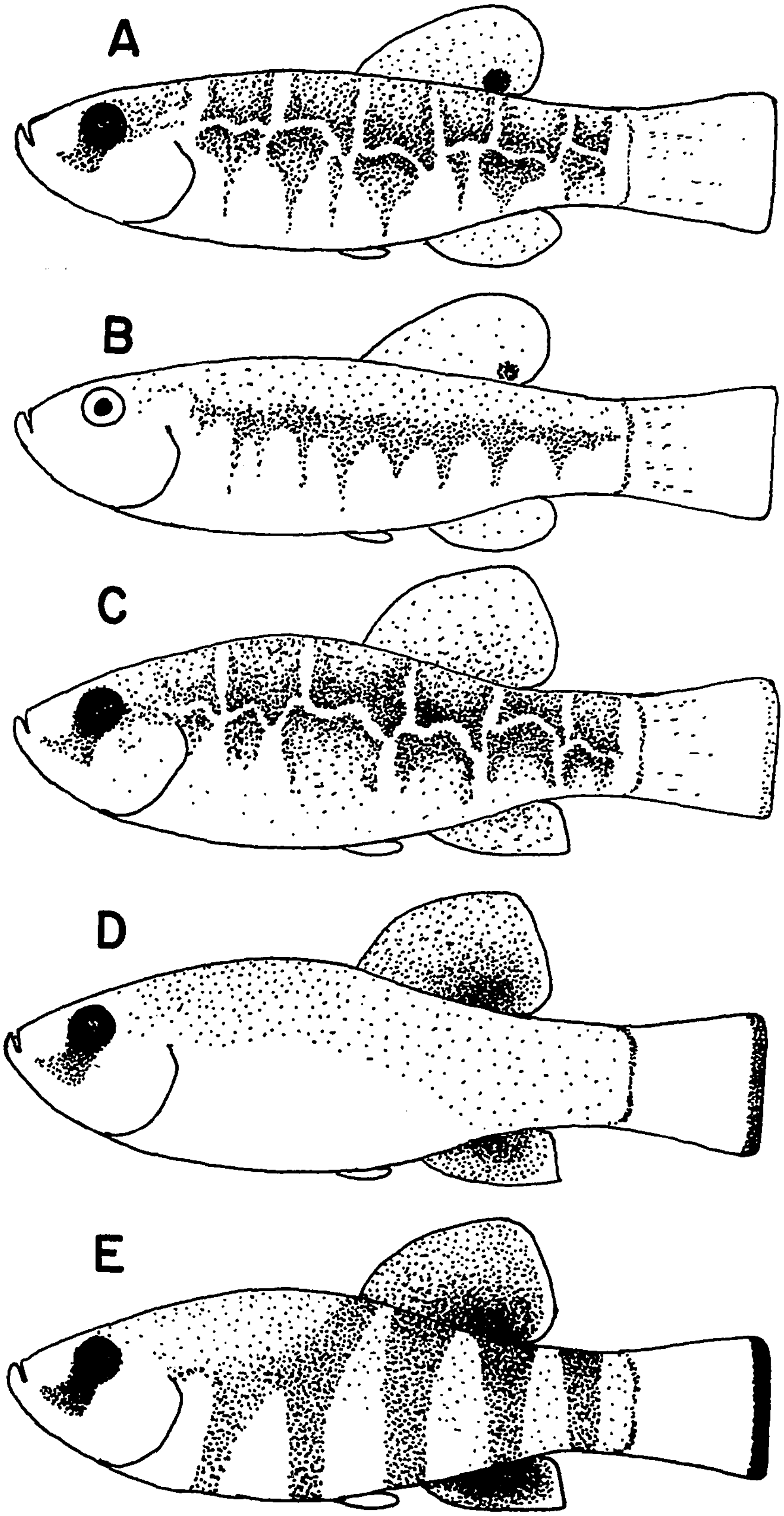

Figure 11. Some typical color patterns of *Cyprinodon rubrofluviatilis*. (A) Blotched female, (B) Lateral Band female, (C) Blotched male, (D) Blue Nape male, (E) Vertical Bars male. The black iris is variably present or absent in Blotched and Lateral Band.

The black iris is the only chromatic element exhibited in all of the color patterns (Table 2). As developed below, the variable occurrence of the black iris in certain patterns and its constancy in others serves as a sensitive clue to the internal motivational state of the fish. The names of those patterns in which the black iris is variable are suffixed with "-black iris" when the black iris is present and "-silver iris" when it is absent.

Rate of Color Change

The purpose of this section is not to give accurate measures of the rapidity of the various color changes, but rather to convey perspective by indicating the general nature of the changes.

Small changes in ground coloration that caused the fish to blend into the background were frequently seen. These changes seemed to be automatic adjustments in response to changes in substrate color and although they were apparently initiated as soon as the new substrate was encountered, a definite time-lag (e.g., seconds) was required for maximum effect.

As noted for Badis badis (Barlow, 1963) and the ten-spined stickleback, Pygosteus pungitius (Morris, 1958) the rate of color change in Red River pupfish usually lagged behind changes in behavior. Males often resumed territorial defense within seconds after being frightened, and sometimes attempted to spawn with other fish prior to attaining aggressive color. The following describes the change from Blanched to Vertical Bars in a male that was frightened as food was being deposited

Table 2. Component parts of the color patterns in male and female pupfish.

	Color Patterns									
Components	Blanched		Blotched		Lateral Band		Mottled		Blue Nape	Vertical Bars
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♂
Ocellus		X		X		X		X		
Black iris	X*		X*	X*	X*	X*	X*	X*	X	X
Teardrop			X*	X*	X*	X*	X*	X*	X	X
Dorsal blotches			X	X			X**	X**		
Lateral blotches			X	X	X**	X**				
Blue nape									X	X
Yellow venter									X	X
Yellow paired fins									X	X
Black caudal band									X	X
Black dorsal fin									X	X
Black anal fin									X	X
Vertical bars										X

* = variable, may be present or absent
** = modified (explained in text)

into the aquarium. The numbers represent time in minutes after I retreated behind the aquarium shroud.

00. Silvery iris, fins hyaline, dorsum light brown, no teardrop, no blotches or bars. Behavior consists of "freezing" on the bottom.

05. Black iris, fins dusky; resumed territorial defense at 01.

12. Dorsum faintly mottled, sides with faint bars, dorsal fin darkening, faint blue wash over caudal peduncle and high on sides of the body. Yellow returning on face, pectorals, and abdomen.

17. Dorsum dark, vertical bars present, yellow more extensive, blue iridescence intense and more extensive.

Males attempting to establish territories in aquaria were frequently chased from their areas when social structure was being established. In these instances, the aggressive color pattern persisted in the displaced male for a short time and then disappeared. If the male then resumed territorial defense, there was a definite time-lag between the resumption of aggressive behavior and the development of the aggressive color pattern.

Color Patterns of Fright and Concealment

It was not surprising that the color patterns of fright and concealment overlap considerably. A frightened fish might assume a concealing pattern when it goes into hiding. In such instances, concealment is presumably motivated by fright. On the other hand, because concealment is of general adaptive value, concealing patterns might be expected at times when fright is at a low level.

The most immediate color response of frightened pupfish was a general paling of the body. In extreme situations (e.g., fish in panicky flight due to extra-aquarium disturbance), all body markings disappeared, forming the Blanched coloration. Fright pallor is common among fishes (cf., McAlister, 1958; Barlow, 1963; and Odiorne, 1957).

Blanched was commonly seen in aquarium-pupfish that were heavily beleaguered by dominant males. These fish tended to rest with the body canted "head up", tail touching the bottom. Blanched fish were often tolerated by territorial males, perhaps because of their subdued movement and coloration. In such situations Blanched may function as "appeasement coloration".

It is difficult to assess the importance of Blanched for pupfish in nature. Its significance as an appeasement response is especially questionable. Fish dominated in aquaria would have more freedom to swim to areas that are devoid of dominant males in natural conditions. Blanched fish frequently swam around near the surface of aquaria and "fluttered" up and down the aquarium walls; this activity would carry them to other areas in natural conditions. Furthermore, as Cox (1966) noted for C. macularius, when territorial males were removed from aquaria, subordinates often assumed dominance over the vacant territories--suggesting that, in nature, the subordinates would have established territories in unoccupied areas. Blanched colors may be more important for subordinates where the streams have become intermittent, concentrating the fish into small volumes of water; but even then there may be considerable opportunity for escape from aggressive males, eliminating the need for appeasement. Okaichi et al (1958) noted that the color patterns of subordinate file fish, Stephanolepis cerrhifer, in crowded aquaria were not seen in a more spacious aquarium where there was no dominance-subordinance relationship. Such observations indicate that some color patterns are "laboratory artifacts" and, as such, may have little relevance to natural conditions.

Blotched was the predominant color pattern in Red River pupfish. It was prevalent in juveniles, females, and non-territorial males when feeding on the bottom, schooling, and resting during the day, in spawning females, fish hiding in darkened places, and in all pupfish, including territorial males, when sleeping.

The Blotched pattern seems cryptic in function, serving to disrupt the body outline. The effect is enhanced by the offset positions of dorsal and lateral blotches. The black iris and teardrop may function similarly, but more specifically, they probably serve to obliterate the outline of the eye as described by Cott (1940) for animals in general. Another element of concealment is the capacity for the pupfish to alter its background color so as to blend with the substrate color.

The Lateral Band pattern was seen in natural and in aquarium situations. In fish swimming several inches off the bottom, the dorsal blotches usually became indistinct, and the lateral blotches remained separate or fused to varying degrees, forming an irregular lateral band. The Mottled pattern is not well understood, but it was sometimes seen when fish were swimming off the bottom. Presumably, these patterns are more cryptic in pelagic surroundings while Blotched is more effective in benthonic surroundings.

Concealment mechanisms that disrupt the body outline and cause the fish to blend with the substrate color would have particular selective value for shallow-water fish that are subject to predation by wading birds and other predators that search for prey from above. Klopfer (1962) and others warn against the tendency to ascribe importance to presumed concealment mechanisms before the perceptive abilities of the predator(s) are known. Mechanisms that conceal pupfish from humans may be more or less effective, given the visual capacities of the usual predators. Sumner (1934, 1935a, and 1935b) has provided evidence that the concealment coloration of *Gambusia affinis* makes them less vulnerable to certain predators. Thus, it is probably safe to assume that the apparent concealment patterns of Red River pupfish are variably effective, depending on the abilities of the predator species.

Schools of pupfish are basically monochromatic. Parr (1931) observed that sexual differences were suppressed in the individuals of certain dichromatic species (e.g., *Gasterosteus aculeatus*) while schooling. Although it is not clear, there is presumably some need for schooling fish to be similar in appearance. Perhaps it is related to a need for the individuals to be closely integrated as a functional whole. Williams (1966) observed that a fundamental motivation for schooling might be a tendency for individuals to hide among conspecifics, in which case conspicuous aggressive color patterns would be disadvantageous. A simple explanation is that monochromatism is due to concealment responses on the part of each individual fish to the particular physical situation (e.g., benthonic or pelagic); however, it is possible that it facilitates the operation of the school as a whole, and enables individuals to hide within the school as well.

A phenomenon perhaps related to the absence of aggressive color patterns in schooling males, is the need for a reduction in "individual distance" (Conder, 1949) while schooling. Blotched or Lateral Band may be more amenable to schooling requirements since the conspicuous aggressive coloration might have a disquieting effect on other members of the school that would affect the cohesiveness of the school.

Color Patterns of Aggressive Behavior

Females and non-breeding males. The only component of the color patterns correlated with aggression in females was the black iris. Black-iris females were more aggressive than silver-iris females (Table 3). The former frequently moved toward and displaced conspecifics, and if they were displaced, it was usually by an aggressive male, or by a female which also possessed the black iris. Silver-iris females seldom chased and were readily displaced by other pupfish. The blackened iris occurred in all female color patterns, excepting Blanched.

Aggressive behavior in non-breeding males was similar to that of females. Correspondingly, the color patterns of those behaving aggressively (e.g., defending feeding sites) were similar to the color patterns of aggressive females. This was most frequently observed in nature, since in aquaria such males were held subordinate to males defending

Table 3. Relationship between the occurrence of the black iris and aggression* in female *Cyprinodon rubrofluviatilis* as shown by a series of three-minute observation periods.

Aquarium #1				
	Activity			
Female	Number of chases	Number of times chased	Ratio	Color of iris
#1	1	17	.06	silver
	2	16	.13	silver
	12	1	12.00	silver
	10	16	.63	silver
	1	14	.07	silver
#2	7	5	1.40	black
	8	5	1.60	silver
	2	9	.22	silver
	5	11	.46	silver
	inactive		-	silver
#3	15	7	2.14	black
	4	12	.33	silver
	3	18	.17	silver
	19	14	1.36	black
	1	21	.05	silver
Aquarium #2				
#1	24	5	4.80	black
	36	1	36.00	black
	32	3	10.67	black
	26	5	5.20	black
	19	3	6.33	black
#2	5	7	.71	silver
	5	17	.29	silver
	0	20	-	silver
	3	11	.27	silver
	3	17	.18	silver
#3	1	11	.09	silver
	0	22	-	silver
	3	12	.25	silver
	3	21	.14	silver
	1	12	.08	silver

* Aggression expressed as the ratio between the number of chases and the number of times the fish was chased.

breeding territories. Like females, non-breeding males never incorporated the black iris into the Blanched pattern.

Breeding males. Blue Nape and Vertical Bars (Fig. 11) were characteristic manifestations of aggressive behavior in males defending breeding territories. However, Blanched-black iris, a transitional stage between Blanched and Blue Nape, was frequently noted for breeding males in aquaria when the fright level was relatively high due to extra-aquarium disturbance. Males exhibiting this pattern chased intruders hesitantly, frequently stopping on or just off the bottom, body canted "head up", giving their activities a furtive appearance; patrolling was repressed and somewhat erratic.

Early in the transition from Blanched-black iris to Blue Nape, the face, pectorals, pelvics and general lower half of the body become suffused with light yellow, and the dorsum darkens. As the yellow pigment intensifies, blue iridescence appears in the nape region and spreads down the sides and posteriorly to varying degrees. Concurrently, the caudal band widens and darkens, and the dorsal and anal fins darken. The blackened iris is retained and the teardrop develops.

Vertical Bars develops from Blue Nape by the addition of vertical bars. These bars are superimposed on the blue and yellow colors of the sides. The appearance of the bars was somewhat variable; sometimes they began to appear in low intensity Blue Nape, but they usually were not formed until Blue Nape had intensified.

The gradation from Blanched-black iris to Blue Nape to Vertical Bars was correlated with behavioral changes. The change from Blanched-black iris to Blue Nape seemed to signify a decline in the fright level, and increased aggressiveness. Blue Nape males patrolled continuously and were likely to chase an intruder until completely ousting it from the territory. Blanched-black iris males behaved erratically and tended to displace intruders for only short distances, generally without sustained chase. Increased aggressive activity was correlated with the transition from Blue Nape to Vertical Bars. Low intensity Blue Nape was more characteristic of situations in which feeding and patrolling predominated and where there was little or no agonistic activity. High intensity Blue Nape developed during periods of intense agonistic activity, and as aggression persisted, it often evolved into Vertical Bars. In aquaria, smaller males consistently seemed brighter and more inclined to assume Vertical Bars than larger males.

An Interpretation of the Aggressive Color Patterns

The basis of the following discussion is the premise that insight into the function of chromatic characteristics can be gained by referring to the function (real or supposed) of presumably related morphological and/or behavioral adaptations. Behavioral, morphological, and chromatic characteristics have often evolved to achieve the same adaptive ends.

General considerations. The brilliant coloration and intense activity of the territorial male make him a conspicuous part of the environment. The blue iridescence flashes brilliantly as the resident males dart about in territorial activity. Each time a male stops to hover, the yellow-colored pectorals are thrown forward and then flickered back and forth, creating conspicuous alternating flashes of yellow on the two sides of the fish; especially in frontal view. Barlow (1961) noted similar effects for C. macularius. This conspicuousness might serve to attract sexually motivated females, while at the same time it could aid in warding off intruding males and sexually refractive females.

As previously mentioned, smaller males usually seemed brighter and more inclined to form Vertical Bars than larger males. It is tempting to attribute these differential tendencies to different psychological states resulting from the difference in size. Larger males, by virtue of their body size, might be "secure" in territorial possession, while smaller males might require brighter colors and Vertical Bars for increased intimidation--a sort of compensation for small size which makes them more effective at territorial defense. This correlates well with the idea developed below that large size confers advantage in agonistic bouts, and that Vertical Bars functions to increase the apparent size of the fish. Further evidence for this interpretation is that when subordinate males established temporary dominance over a portion of the aquarium, they always assumed Vertical Bars. Here again, is an example of a male in a "precarious" situation, in that he must defend the area against males by which he has been dominated. By increasing the apparent body size, Vertical Bars may enhance the ability of these males to hold more secure males in abeyance.

Simpson (1968) described a situation for Betta splendens that seems analogous to the apparent tendency for pupfish to develop Vertical

Bars in "precarious" situations. In comparing the tail beating behavior (an activity of threat display) of "winners-to-be" with that of "losers-to-be", he presented evidence that tail beating may be done to achieve the goal of "partner-with-its-gill-covers-down-again", with winners being less sensitive to this stimulus (tail beating) than losers. In other words, Simpson's "winners" seemed to keep the gill covers erect in spite of increased tail beating in the "loser". The losing Betta would seem to be in the same general predicament as was described for the pupfish in "precarious" situations; that is, under threat by an evidently superior rival. If the pupfish's aggressive color pattern is meant to inhibit overt aggression in the rival, then there may be a tendency for larger males to behave aggressively in spite of the smaller male's aggressive coloration, to which the smaller may respond by increasing the intensity of his coloration and developing Vertical Bars. In both instances the "insecure" fish may be responding to a certain response inertia in the "secure" male that is perhaps a product of inherent superiority.

The phenomenon of Typical Intensity (Morris, 1957) may have been operative in the evolution of the aggressive color patterns in Red River pupfish. Behavior in situations where an animal is in a state of conflicting internal motivations is often interpreted as being the manifestation of both motivations. For instance, in agonistic encounters an animal might be simultaneously motivated by fright and aggression. Such ambivalently motivated animals often exhibit ritualized agonistic displays that seem to consist of fright components and aggressive components (cf., Hinde, 1953; and Tinbergen, 1954). These displays apparently have signal value that inhibits overt attack from rivals. Morris (_l.c._) postulated that it is of value in animals like fishes that such signals become ritualized or rigid in form, so as to prevent signal ambiguity. If a behavior exhibits Typical Intensity, it maintains constant form over a relatively broad range of stimulation intensities. Morris used the term "Typical Compromise" for situations where a typical intensity has developed for ambivalently motivated responses.

Blue Nape and Vertical Bars are apparently exhibited by males under a wide range of relative levels of fright and aggressive tendencies. For example, males that are "secure" in their territories (see above) presumably represent individuals having low fright levels and high aggressive levels (relative to the fright level), whereas males less secure in their territories represent individuals having higher fright levels; however, the two groups exhibit essentially identical aggressive color patterns. This indicates that the aggressive color pattern has a broad motivational base and suggests that it might have arisen as a Typical Compromise.

If this is a valid hypothesis, then it should be possible to detect certain fright components of the aggressive coloration and certain aggressive components. The dark iris and teardrop are usually developed in pupfish that have gone into hiding, where they serve as camouflage; thus, they might represent fright components that are incorporated into the aggressive display. The yellow pigment, blue iridescence, dark fins and caudal band render the fish conspicuous and presumably represent aggressive components of the display--they disappear in fright situations. The vertical bars may also represent aggressive components since, as discussed below, they may function to increase the apparent size of the fish. This scheme accords well with behavior. The black iris and teardrop are the first chromatic elements that develop when territorial males are recovering from fright, whilst yellow pigmentation, blue iridescence and vertical bars return during later stages of the recovery. The black iris and teardrop develop when the fright level is high, while the other chromatic elements of agonistic display develop as the fright level wanes and aggressiveness returns. Barlow (1963) and Baerends _et al_ (1955) have employed the scheme of reciprocally interacting fright and aggression in analyzing color patterns.

Analysis of the function of the black iris. The correlation between social status in aquaria and the presence or absence of the black iris was noted in _C. macularius_ by Cox (1966). He stated, "When a male is dominant the iris of his eye is very dark, probably due to the expansion of melanophores in this area. In contrast, the subordinate male's iris is almost devoid of any black pigment and it appears as a silvery ring around the lens." Barlow (1961) noted that males of the same species in "breeding colors" have a "black eye". Although both authors mentioned female aggressiveness, neither noted any correlation between the occurrence of the black iris and female aggressive behavior, indicating that either it simply went unnoticed, or else there was no such relationship. As previously noted, the occurrence of the black iris is associated with aggressive behavior in non-breeding males and in females of _C_. _rubrofluviatilis_, as well as in breeding males, while less aggressive individuals display a silver iris.

The black iris may be manifested in any of the color patterns of male pupfish, and in any of the female patterns excepting Blanched (Table 2). Presumably, the black iris is an element of camouflage when pupfish are sleeping or hiding motionless in darkened areas. However, when swimming freely in open areas, this mark (and the associated teardrop) becomes conspicuous; it is at these times that black-iris fish behave aggressively.

Barlow (1961) stated for _C_. _macularius_ that the black iris may function as clues in the orientation of fighting behavior among breeding males. This could be true for reproductive _C_. _rubrofluviatilis_ males, but it would not seem operative in females and non-breeding males, since their aggressive encounters are brief and consist of little more than one moving toward another and displacing it. The black iris may create increased intimidation of conspecifics by signifying that the individual has tendencies toward overt attack; thereby facilitating the displacement of other fish without the necessity of a fight.

The observation that frightened breeding males in aquaria sometimes display a black iris when otherwise blanched, while this is not seen in females and non-breeding males, may be explained as being the result of the high level of aggressiveness in reproductive males. In fact, such males sometimes behave aggressively in the blanched condition before the black iris appears. Territorial males have a low threshold for aggression, and a correspondingly low threshold for the development of the black iris, further evidence for the belief that aggression and the black iris are related through common motivation.

Function of the Vertical Bars pattern in agonistic behavior. Raney et al (1953) noted for *C. variegatus* that males tend to be slightly larger than females, but from the appearance of active fish, the size difference seemed greater than actual measurements demonstrated. They concluded, "The illusion of larger size in males is chiefly due to the greater activity of the male." From my experience, the same could be said for *C. rubrofluviatilis*. However, intuitively, it seems that the Vertical Bars pattern may function to specifically make the male appear larger in lateral profile. Comparison of Vertical Bars with the other male color patterns (Fig. 11) indicates that Vertical Bars may function to emphasize the depth of the fish, whereas counter-shading detracts from the depth of the profile in the other patterns.

As discussed elsewhere, male pupfish exhibit behavioral and morphological characteristics which seem aimed at increasing the size of the aggressive image presented to rivals during agonistic bouts. A usual preliminary to actual physical combat between two males is that each presents his lateral aspect toward the other. Hypothetically, lateral display might represent a "sizing up" period with the ultimate outcome (i.e., if the bout is terminated before or after a fisht ensues) being partly dependent upon the apparent size of each fish as seen by the other.

Another postulate developed elsewhere is that large size confers advantage in agonistic encounters, in that it may inhibit attack from rivals. If this is so, then behavioral or structural elements that increase the apparent body size could represent evolutionary parallels aimed at inhibiting attack from conspecifics. Whether the adaptive value lies in inhibiting attack or not, the fact remains that certain characteristics have evolved which increase the size of the profile. Assuming that this tendency exists because of some derived adaptive value, then any complementary chromatic factor that enhances the "desired" effect might have adaptive premium.

A number of hypotheses may explain how Vertical Bars might enhance behavioral and structural tendencies toward increasing the size of the aggressive image presented by territorial males. Two reasonable hypotheses are: (1) the bars, by extending unbrokenly between the upper and lower limits of the profile, could emphasize the body depth, and (2) the pigmentation of the paired and median fins could accentuate the effect produced by spreading the fins (i.e., the broadening of the profile) during lateral display.

Color Patterns of Reproductive Behavior

The color patterns of males engaged in courtship and spawning were the same as those described for aggression. Aggressive males not engaged in courtship often displayed a low intensity Blue Nape pattern. The colors generally intensify and Vertical Bars may develop during periods of active courtship; one male that had been patrolling and feeding in his territory changed from low intensity Blue Nape to Vertical Bars after 45 seconds of courting a female. Vertical Bars apparently reflects the highest levels of both aggressive and sexual motivation in the male. Barlow (1962b) in discussing the observation that the "Black" pattern of Badis badis is indicative of both high aggressive and high sexual motivation, stated: "Actually there need be no contradiction. Black males, when behaving sexually are extremely aggressive." The same could be said for pupfish. Courting males were frequently observed to leave the female and vigorously chase fish that ventured too close. Blotched males were sometimes observed courting females in nature. In these instances, they exhibited a black iris, indicating that aggressiveness is an important motivational component in the courting male.

The effect postulated for Vertical Bars, increasing the size of the profile, may have value in courtship as well as in agonistic encounters. During courtship the male performs various movements (e.g., zigzagging and looping) during which the female has opportunity to view him in profile. Thus, any advantage accrued by presenting a large profile, would lend additional adaptive value to the hypothetical effect proposed for Vertical Bars. Indeed, large size seemed to confer advantage in reproductive activity, for larger males seemed more effective in courtship than smaller males. Males as small as 20 mm, TL, were often observed courting females in nature as well as in aquaria, but they seemed less successful than larger males. Females tended to swim through such a male's territory as though unaffected by his courtship antics. Perhaps large size creates a greater overall stimulus situation than does smaller size because of the greater surface area for the display of nuptial colors, but it may be that the female is simply more responsive to the image "large male", than she is the image, "small male".

Spawning females exhibited a Blotched pattern with a silvery iris, and the teardrop was usually absent. The absence of the black iris in spawning females indicates that they present a non-aggressive image to the male. Marks that are readily visible correlates of aggressive behavior might provoke the male to attack. The absence of the black iris might be an appeasement response that helps reduce the male's aggressiveness enough to allow successful courtship.

Regarding other cyprinodontines, Foster (1967) stated: "There is no fading of the color of the [*Cyprinodon variegatus*] females prior to spawning..., a behavioral difference between this species and the flagfish, for females of *Jordanella* undergo a very pronounced fading just before approaching a territorial male to solicit spawning." The loss of the black iris in *C. rubrofluviatilis* and fading of the body coloration in *Jordanella* may be functionally homologous.

CHAPTER VI

INTERSPECIFIC RELATIONSHIPS

Species Associations

During this study 29 species of fishes were collected within the range of *C. rubrofluviatilis*. The range of dissolved solids at which each species was collected and the frequency at which it occurred with pupfish are shown in Table 4. The data for 1963 and 1969 show somewhat similar results except for the high occurrence of *Hybopsis aestivalis* and *Pimephales promelas* with *C. rubrofluviatilis* in the former. Based on the relative frequencies of occurrence of certain species, it appears that Lindsey and Bates collected more in the larger streams than was done in the present study. The five most frequently encountered species in the 1969 collections were *Fundulus zebrinus* (= *kansae*), *Hybognathus placita*, *Notropis lutrensis*, *Notropis bairdi*, and *Gambusia affinis*. These five were the most frequent concomitants of *C. rubrofluviatilis* in the collections, and their occurrences were analyzed in detail, as was that of centrarchids, treated as a single entity.

There were three major associations among the fishes considered in Table 5: (1) *N. lutrensis*-*G. affinis*-centrarchid, (2) *H. placita*-*N. bairdi*, and (3) *C. rubrofluviatilis*-*F. zebrinus* (herein referred

Table 4. Fishes taken within the geographical range of *Cyprinodon rubrofluviatilis* in 59 collections made in 1969 and 66 collections made by Lindsey and Bates (from UOMZ records) in 1963. Those species taken in the twenty-nine 1969 collections containing *rubrofluviatilis* are arranged in order of decreasing frequency in those collections. N = total number of collections containing the species; % = per cent frequency of occurrence in collections containing *rubrofluviatilis*; ppt = range of dissolved solids at which the species was taken in parts per thousand.

Species	1969			1963	
	ppt	%	N	%	N
Cyprinodon rubrofluviatilis	0.42-51.70		29		29
Fundulus zebrinus	.28-43.22	90	44	93	44
Hybognathus placita	1.20-21.72	52	26	66	41
Notropis bairdi	.40-19.66	52	28	66	38
Notropis lutrensis	.28-13.00	52	44	55	49
Gambusia affinis	.34-19.66	45	33	45	41
Lepomis cyanellus	.34-19.66	34	31	21	20
Hybopsis aestivalis	1.20-19.60	17	13	45	31
Pimephales promelas	.34-13.04	14	10	38	30
Lepomis megalotis	.40- 7.00	14	15	7	13
Notropis percobromis	.66- 9.50	10	9	21	18
Notropis potteri	4.20-19.72	10	4	10	13
Pimephales vigilax	.34- 2.80	10	11	21	17
Carpiodes carpio	.86- 5.80	10	8	10	19
Lepomis humilis	4.60-11.00	10	8	14	12
Ictalurus melas	.40- 8.20	7	9	0	2
Micropterus salmoides	.42- 8.20	7	6	3	2
Dorosoma cepedianum	1.44- 5.80	3	2	10	14
Notropis stramineus	.46- 1.74	3	2	7	9
Cyprinus carpio	.46-12.48	3	5	0	5
Lepomis microlophus	2.80	3	1	0	0
Lepisosteus osseus	4.50- 9.50	0	2	7	2
Notropis venustus		0	0	0	1
Notropis buchanani		0	0	0	2
Notemigonus chrysoleucas	2.40	0	1	0	0
Phenacobius mirabilis	2.10-10.00	0	3	10	9
Ictiobus sp.	6.50	0	1	0	1
Ictalurus punctatus	.86- 9.50	0	3	10	19
Ictalurus natalis	.40	0	1	3	2
Noturus nocturnus		0	0	0	2
Pomoxis annularis	.24	0	1	0	2
Percina phoxocephala		0	0	0	1
Aplodinotus grunniens		0	0	0	1

Table 5. Results of 2 X 2 contingency tables, comparing the presence or absence of centrarchids and six species of fishes in 59 collections made in 1969 (A), and 67 collections made in 1963 by Lindsey and Bates (B).[*]

(A)

Cyprinodon rubrofluviatilis						
n.s.	Notropis bairdi					
--	n.s.	Notropis lutrensis				
n.s.	++	n.s.	Hybognathus placita			
+	+	n.s.	n.s.	Fundulus zebrinus		
n.s.	n.s.	++	-	+[**]	Gambusia affinis	
--	n.s.	++	n.s.	n.s.	++	Centrarchidae

(B)

Cyprinodon rubrofluviatilis						
n.s.	Notropis bairdi					
-	n.s.	Notropis lutrensis				
n.s.	++	n.s.	Hybognathus placita			
++	n.s.	n.s.	n.s.	Fundulus zebrinus		
n.s.	n.s.	+	n.s.	n.s.	Gambusia affinis	
-	n.s.	++	n.s.	n.s.	n.s.	Centrarchidae

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[*] ++ = highly significant positive relationship ($p < .01$); + = significant positive relationship ($p < .05$); -- = highly significant negative relationship; - = significant negative relationship; n.s. = nonsignificant Chi-square.

[**] Chi-square = 3.88; significance at the 5% level requires a value of 3.84.

to as the rubro-zebrinus association). The criterion for determining species associations was that two species occurred together more often than separately, with a probability of less than .01 (in Table 5-A or 5-B) that the observed association was a chance occurrence. Again, the 1963 data gave results similar to those obtained from data collected in 1969, the major discrepancy being the G. affinis-centrarchid relationship. In 1963, affinis and centrarchids occurred together in 22 collections and separately in 29; in 1969 they occurred together in 27 and separately in 16. They were placed in the same association because of the obvious association of both with N. lutrensis.

Considerable overlap existed among the three associations. They frequently occurred together, and a particular species association usually occurred with individuals of another association. However, with respect to the dissolved solids content of the water, the three associations tended to be spatially segregated (Fig.12). The rubro-zebrinus combination was predominant at higher dissolved solids, being the only association found in waters above 20 ppt. The wide range of dissolved solids supporting rubrofluviatilis and zebrinus (Table 4, Fig. 12) reflects the wide physiological tolerances of the two species. The placita-bairdi combination was the most frequent association at the mid-range of dissolved solids, while the lutrensis-affinis-centrarchid complex was predominant at the lower dissolved solids (Fig. 12).

That the dissolved solids content of the water is a significant factor limiting the distribution of fishes within the range of the pupfish is further indicated by Figure 13. There was a significant

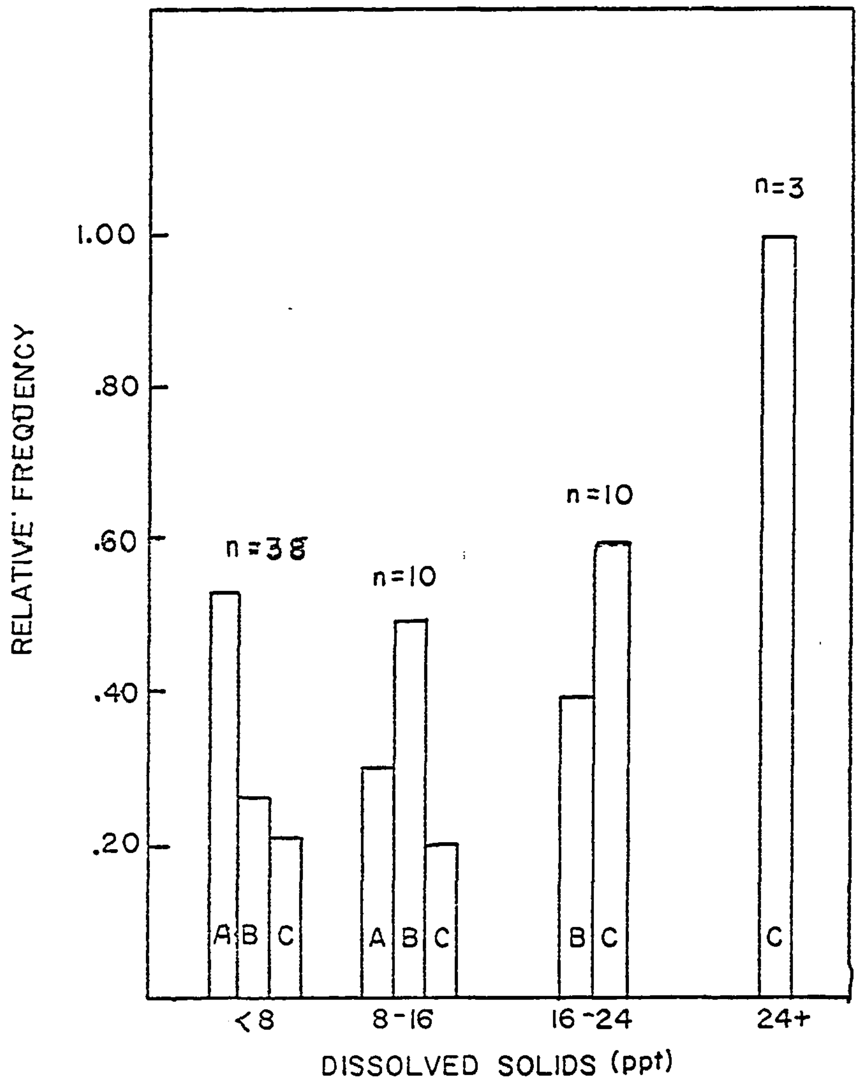

Figure 12. Relative frequencies of occurrence of the three major species associations within the range of *Cyprinodon rubrofluviatilis* versus total dissolved solids at the collection sites. Relative frequencies were calculated by dividing the number of occurrences of each species association by the sum of the occurrences of the three associations at each range of dissolved solids. n = total number of occurrences. A = *lutrensis*-*affinis*-centrarchids; B = *placita*-*bairdi*; C = *rubrofluviatilis*-*zebrinus*.

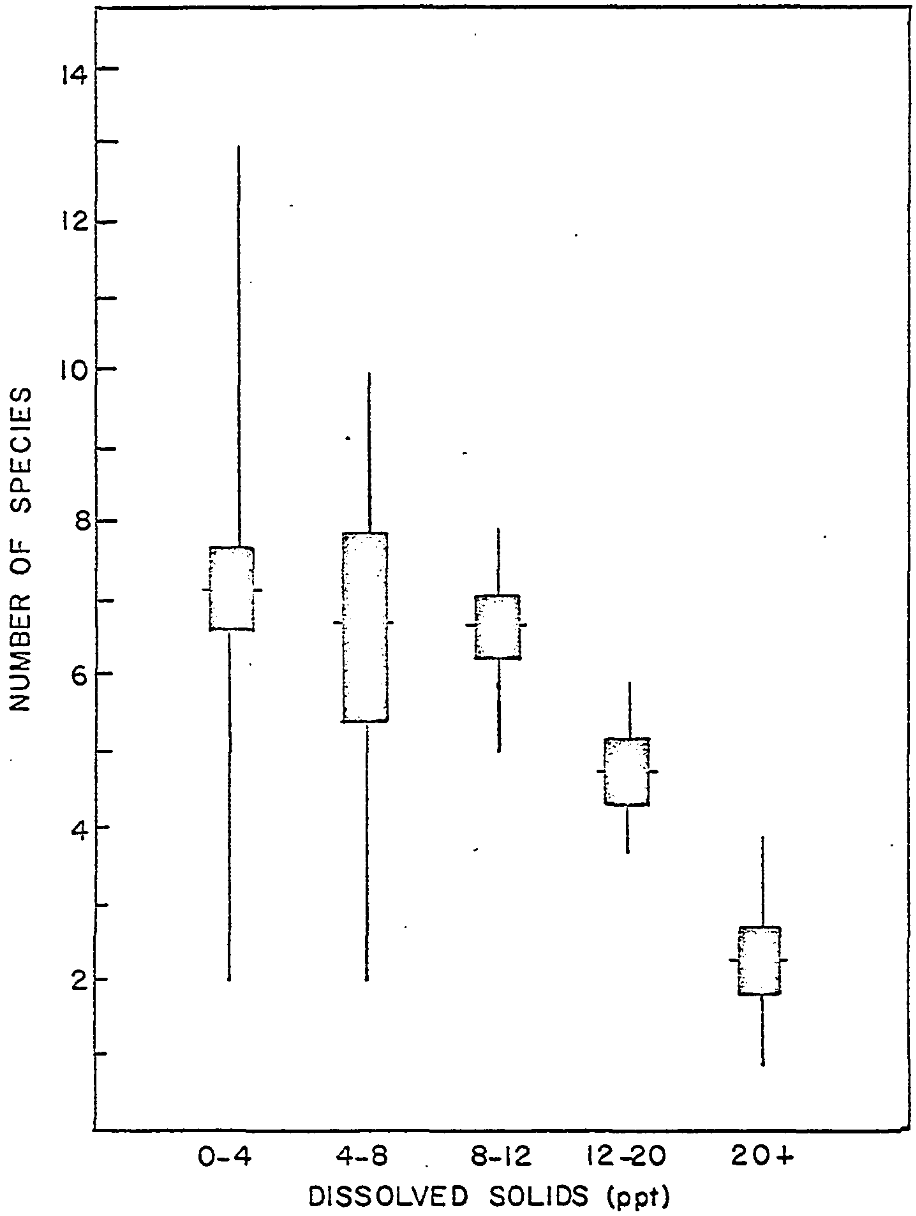

Figure 13. Number of species of fishes taken versus total dissolved solids at each collection site. Vertical lines = range; horizontal lines = means; solid bars = one standard error on each side of the mean.

drop in the number of species taken at dissolved solids higher than 12 ppt.

The declining frequency of the *rubro-zebrinus* combination with decreasing dissolved solids is primarily a reflection of the decreased frequency of occurrence of *C. rubrofluviatilis* at lower dissolved solids. The frequency of *F. zebrinus* was little affected by the dissolved solids content of the water; it occurred in 88 and 72%, respectively, of the collections from waters higher and lower than 12 ppt. Although the pupfish occurred in all (16) collections taken from waters with dissolved solids higher than 12 ppt, it occurred in only 13 (30%) of 43 collections taken at lower concentrations.

Limiting Factors of the Distribution of Red River Pupfish

Martin (1968) stated that, "*Cyprinodon variegatus*, *C. rubrofluviatilis*, and '*C. bovinus*-like' fishes...are not limited in their distribution by Ca^{2+}, Na^+, K^+, or Mg^{2+} or by any combination of these ions in most areas." His conclusion was based primarily on the observation that the majority of the localities in which *Cyprinodon* did not occur had levels of all ions and ion ratios within the ranges at which *Cyprinodon* were found. *Cyprinodon* in general are tolerant of extremes in environmental factors (cf., Barlow, 1958; Lowe, Hinds, and Halpern, 1967; Minckley and Itskowitz, 1967).

As Martin (l.c.) showed for *C. variegatus*, *C. rubrofluviatilis* does not seem limited in its distribution by bottom type, vegetation, or turbidity. The streams supporting pupfish varied from turbid to very clear, from soft mud bottoms to sand and gravel, and from areas with dense growths of vegetation (e.g., filamentous algae and Potamogeton), to others with little or no such growths. The current varied from "slight to moderately swift". Spawning substrate did not seem important in the distribution, for they were observed to spawn on sand, soft mud bottoms, the surface of stones, and within mats of plant debris.

The occurrence, albeit sporadic, of the pupfish in very dilute waters (as low as .42 ppt) as well as at higher concentrations suggest that the dissolved solids content per se does not present an impenetrable barrier to its distribution. I have kept them in freshwater tanks where they produced offspring that grew in size until removed from the tanks three months after hatching.

Pupfish occurred in greater abundance and represented larger proportions of the ichthyofauna in regions of high dissolved solids and low species densities (Fig. 14 and 15). Considering the apparent "indifference" of pupfish (in terms of survival and reproduction) to the chemical nature of the water, it follows that the causative factor of the positive correlation between dissolved solids and the abundance of pupfish may be found in the second correlation; i.e., the negative relationship between abundance and the number of species present.

A partial explanation for the extreme negative correlation between the abundance of pupfish and the number of species present may be that while capable of surviving and reproducing, the pupfish is physiologically less efficient in areas of low dissolved solids. Through competitive exclusion, it may be partially eliminated from such areas

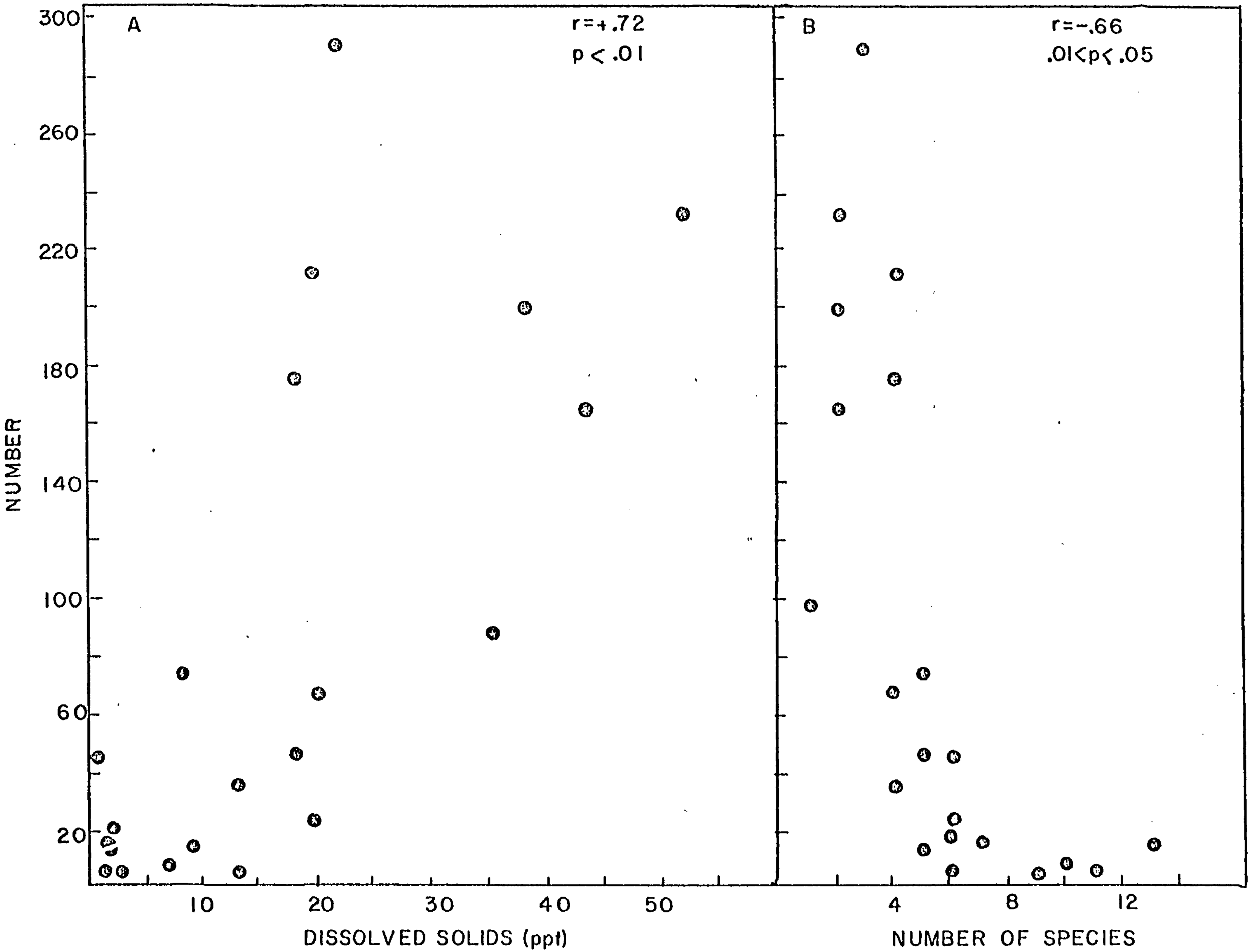

Figure 14. Number of pupfish taken versus (A) total dissolved solids of the water and (B) number of species taken at each collection site. r = product-moment correlation coefficient; p = probability of the correlation. Only those collections containing the pupfish are considered.

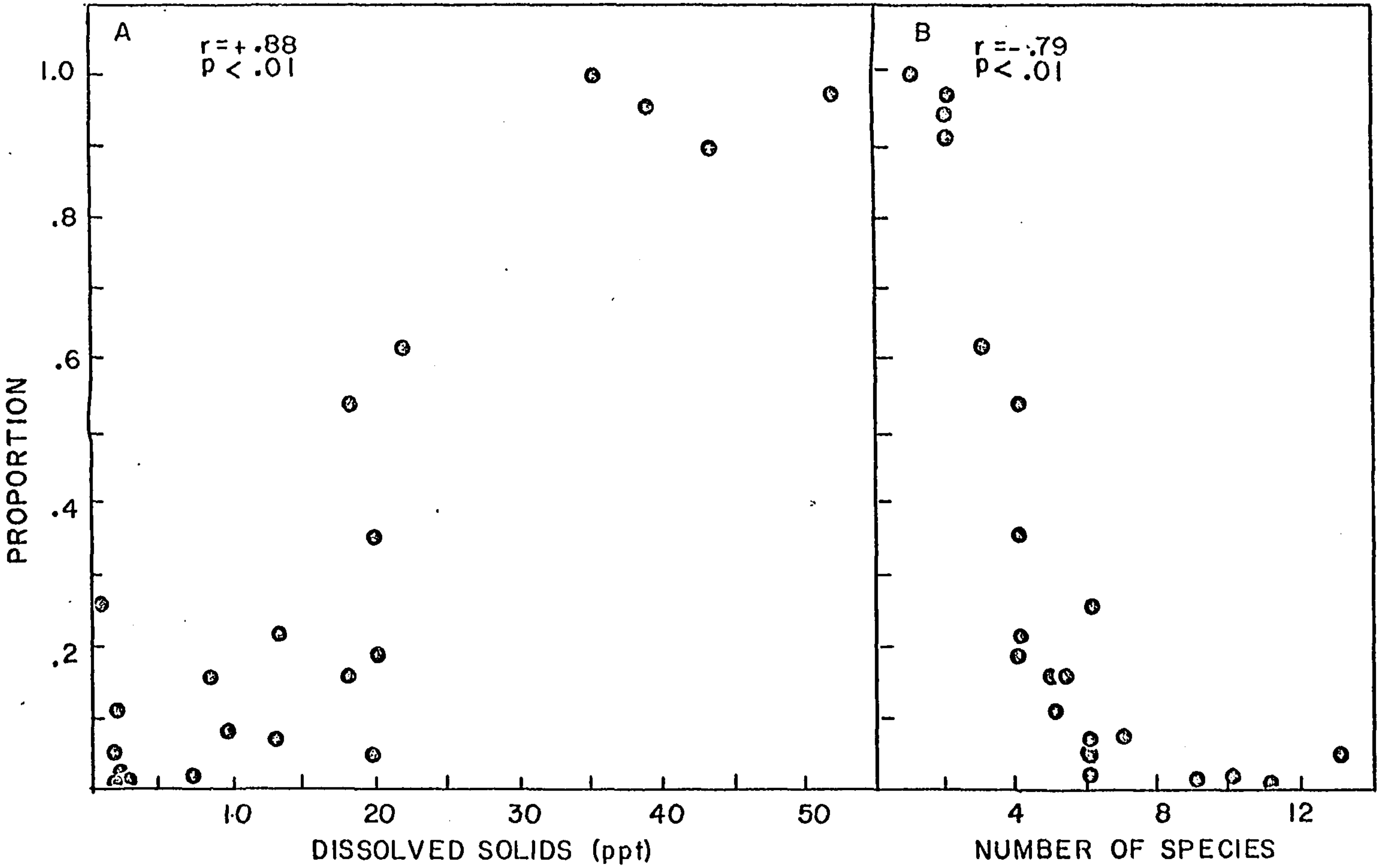

Figure 15. Proportion contributed by pupfish to the total number of fishes taken versus (A) total dissolved solids of the water and (B) number of species taken at each collection site. r = correlation of the angular transformations; p = probability of the correlation. Only those collections containing the pupfish are considered.

by freshwater species functioning at maximum efficiency. Kinne (1960) found that, although *C. macularius* occurred in freshwater as well as at high salinities, it was metabolically less efficient in waters lower than 15 ppt.

Another partial explanation may be that by being adapted to a broad ecological niche, the pupfish is confronted with a number of competing species at low dissolved solids. Kaill (1967) stated: "The behavior of *Cyprinodon variegatus* is flexible. It seems to be made up of components assembled in a non-stereotyped manner which are then modified to the demands of a particular habitat." Examples of "broad" adaptation in *C. rubrofluviatilis* seem to be (1) it is omnivorous in its food habits; (2) although primarily restricted to the bottom, it is frequently seen feeding at all depths in both shallow and deep water; (3) the reproductive season is prolonged and spawning occurs at a wide range of water temperatures; and (4) breeding males defend territories in a wide variety of situations. These factors would bring pupfish into competition with a number of "freshwater" species within its range, each of which might be more specialized in habit, and thereby better able to compete in areas supporting a varied ichthyofauna.

Of the species considered in Table 5, the most likely ones effecting exclusion of pupfish from "freshwater"are *N. lutrensis* and centrarchids. Mutual exclusion between these fishes and pupfish is indicated by the negative Chi-square values obtained from contingency-table-analysis of presence-absence data (Table 5). However, although such analyses may be used to measure the degree of positive or negative association, they are not measures of competition (Hurlbert, 1969). Negative associations between fishes could be manifestations of habitat selection. Pupfish may simply "select" waters of higher dissolved solids while "freshwater" species select lower dissolved solids; this would eliminate the possibility of competition being a major limiting factor in pupfish distribution.

Interrelationships Between Pupfish and *Fundulus zebrinus*

The Red River pupfish was more abundant than *F. zebrinus* at higher dissolved solids while the reverse was true at lower dissolved solids (Fig. 16). This was due primarily to the positive relationship between the abundance of pupfish and the dissolved solids content of the water (Fig. 14).

Although there was a slight negative correlation between the abundance of *F. zebrinus* and the number of species collected, the observed relationship was not significant (Fig. 17). Unlike the pupfish, the abundance of *zebrinus* is apparently not greatly affected by the number of species present. *F. zebrinus* may be adapted to a relatively narrow ecological niche, rendering it more efficient than the pupfish at low dissolved solids where the available resources are usually partitioned between the species of a varied ichthyofauna. On the other hand, since *C. rubrofluviatilis* seems to be adapted to a broad ecological niche, it may be more efficient than *F. zebrinus* in utilizing niches left vacant by the absence of other species at higher dissolved solids.

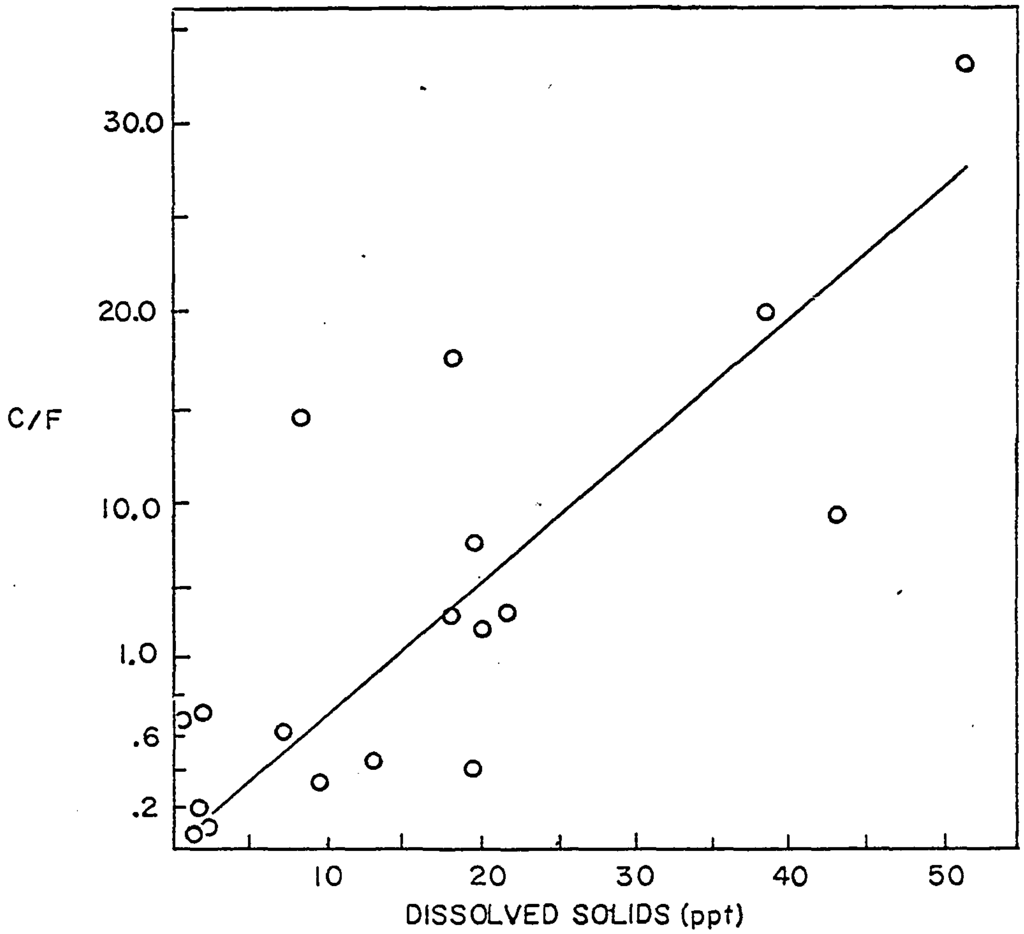

Figure 16. Ratio (C/F) of the number of pupfish to the number of Fundulus zebrinus versus total dissolved solids content of the water at each collection site, considering just those collections containing both species. The regression line was fitted by eye.

The following evidence suggests that the pupfish exerts a negative effect on the abundance of *F. zebrinus*: (1) Like the pupfish, *zebrinus* represented larger proportions of the ichthyofauna in areas of low species density. However, this is shown only if those collections containing the pupfish are excluded from consideration. At low species densities, the proportion of *zebrinus* was depressed in collections containing pupfish (Fig. 18). (2) The abundance of *zebrinus* in those collections containing pupfish showed a slight negative correlation with dissolved solids (Fig. 17). This was correlated with an increase in the abundance of pupfish with increasing dissolved solids.

A number of factors might account for the depression of the abundance of *F. zebrinus* at higher dissolved solids. Among these are intrinsic factors such as habitat preference and physiological effects of dissolved solids, and extrinsic factors, such as interspecific competition and predation. Regarding habitat preference, Cross (1967) stated that it, "inhabits shallow streams that have sandy bottoms and are highly alkaline or saline." Such habitats are common in the areas of high dissolved solids within the range of the pupfish. *F. zebrinus* is noted for its euryhaline capabilities (cf. Meier and Fleming, 1962), and, as previously noted, the frequency of *zebrinus* seems to be little affected by the dissolved solids content of the water; in fact, in the present study it occurred more frequently at higher than at lower dissolved solids. Consequently, the observed negative relationship between the abundance of *F. zebrinus* and the dissolved solids content of the water does not seem to be due to an intrinsic physiological

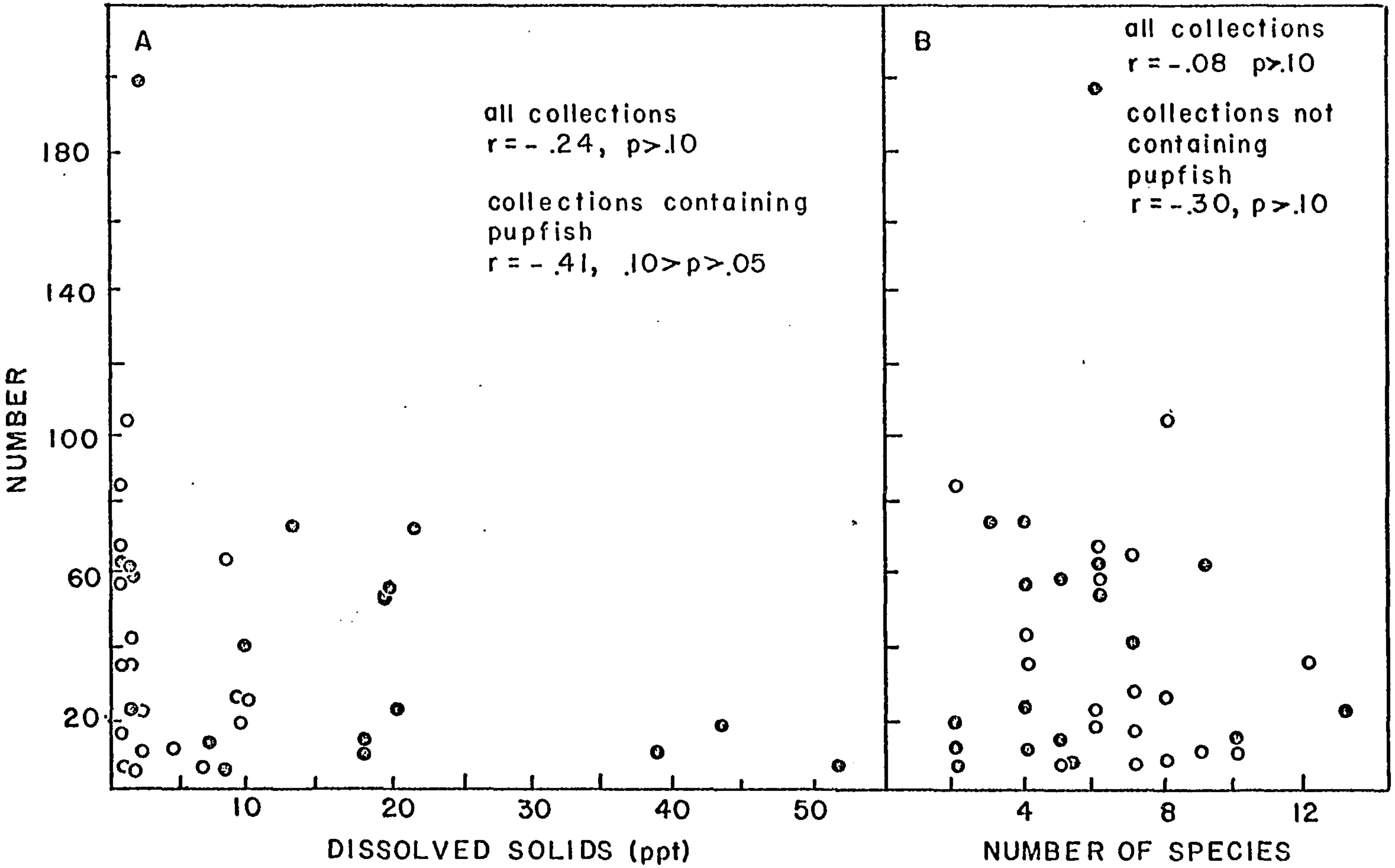

Figure 17. Number of Fundulus zebrinus taken versus (A) total dissolved solids content of the water and (B) number of species at each collection site. Only those collections containing zebrinus are considered. Open circles = collections not containing Red River pupfish; closed circles = collections containing the pupfish. r = product-moment correlation coefficient; p = probability of the correlation.

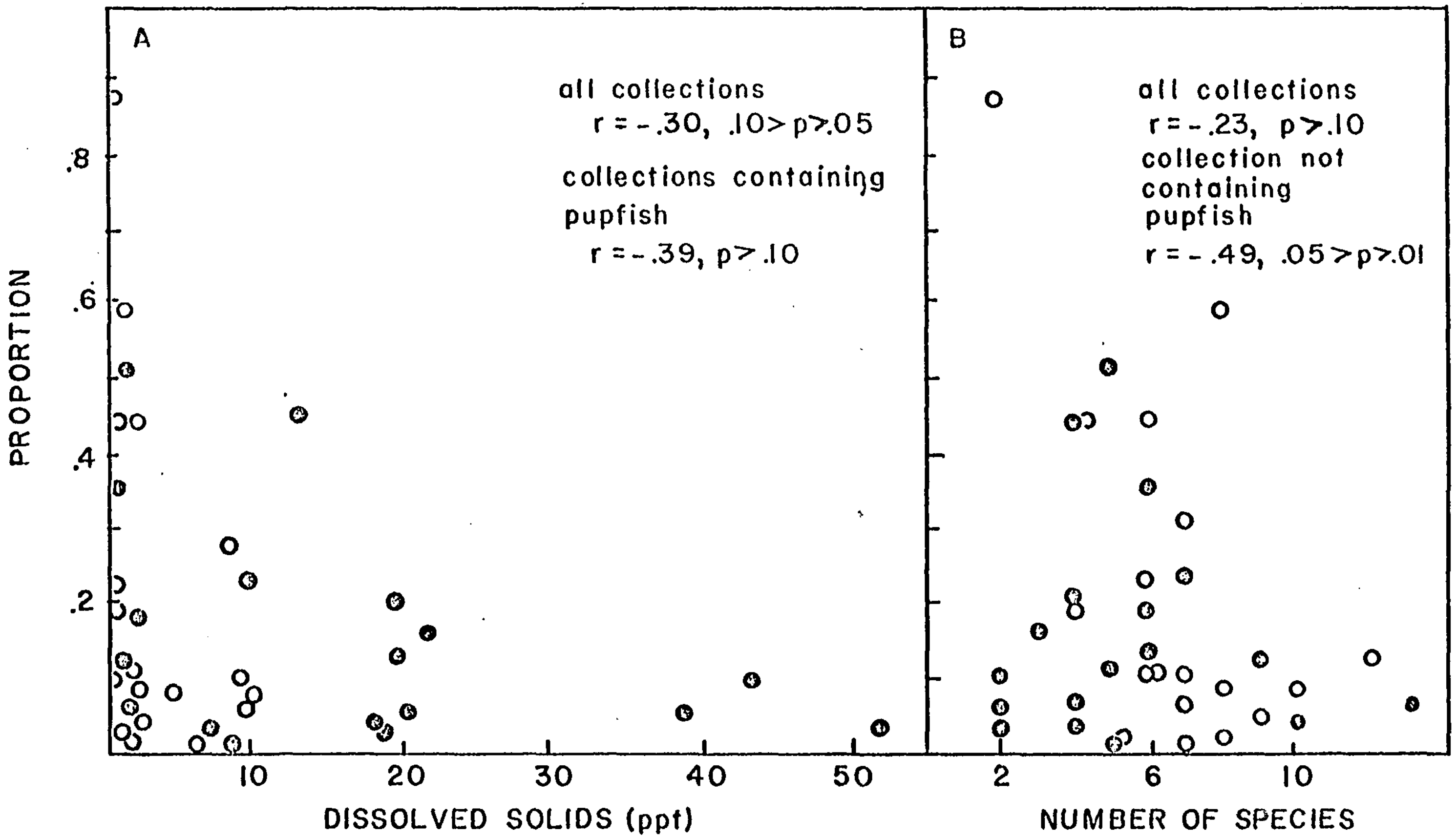

Figure 18. Proportion contributed by Fundulus zebrinus to the total number of fishes versus (A) total dissolved solids content of the water and (B) number of species taken at each collection site. Only those collections containing zebrinus are considered. Open circles = collections not containing Red River pupfish; closed circles = collections containing the pupfish. r = product-moment correlation coefficient; p = probability of the correlation.

inefficiency at higher dissolved solids.

Of the extrinsic factors, interactions with pupfish seem to be the most likely ones effecting a depression of the abundance of *F. zebrinus* at higher dissolved solids. These two species were the only species present at dissolved solids above 20 ppt, and they were the most abundant fishes at dissolved solids above 12 ppt. The major avenues of predation on *zebrinus* may be intraspecific predation on the young and eggs and egg-predation by the pupfish. The latter source of predation may be significant in view of the bottom-feeding habits of pupfish and the fact that, in this study, *zebrinus* were seen spawning on the bottom in areas where pupfish were feeding actively.

Fundulus zebrinus and Red River pupfish exhibit some degree of behavioral similarity, and it is possible that, through competition, the latter exerts partial exclusion of *zebrinus* from areas of high dissolved solids. As *zebrinus* is primarily carnivorous (Minckley and Klaassen, 1969), it ingests more animal foods than the pupfish, but there is considerable overlap in the kinds of animal foods taken (pers. obs.). There is some vertical segregation of the feeding populations, with *zebrinus* occurring more frequently at the surface and pupfish more near the bottom; however, *zebrinus* were frequently observed feeding on the bottom in a manner similar to digging and nipping as exhibited by the pupfish, and the pupfish, especially juveniles and females, were commonly seen feeding at the surface.

F. zebrinus were seen spawning on a number of occasions in areas where pupfish were abundant during this study; they, like pupfish, are bottom spawners (Koster, 1948) and there seems to be a significant amount of competition for spawning sites between the two species. I observed numerous agonistic bouts between *zebrinus* males attempting to defend breeding territories and territorial pupfish males. Being more motile in courtship, *zebrinus* require more space for effective courtship than does the pupfish. This often resulted in confrontations with several territorial pupfish males in succession when *zebrinus* were behaving sexually. Male pupfish were observed to physically disrupt the spawning act of *zebrinus* by ramming into pairs that were S-shaping on the bottom. The significance of such interactions is heightened by the fact that *zebrinus* has a prolonged spawning season that is completely encompassed by that of *rubrofluviatilis*. Minckley and Klaassen (1969) indicated that, in Kansas, *zebrinus* began spawning in May and that all females were spent by August. I have observed *zebrinus* spawning in southwestern Oklahoma as early as March 28 and as late as October 27. In Oscar Creek on July 29, 1969, male pupfish were observed to interfere with almost every courtship attempted by a particular *F*. *zebrinus* male.

The pupfish is, in general, more aggressive than *zebrinus*. In areas of abundant pupfish, feeding *zebrinus* were frequently observed being chased from place to place by territorial male pupfish, and in areas not occupied by such males, they were frequently confronted with aggressive members of the feeding population. It seems likely that competition for food and spawning sites added to the energy extracted from *zebrinus* by pupfish aggression may account for the observed depression of the *zebrinus* population in areas where pupfish were abundant.

CHAPTER VII

SUMMARY

1. The Red River pupfish, Cyprinodon rubrofluviatilis, occurs in the upper reaches of the Red and Brazos rivers. The streams within the range of its distribution can be generally characterized as having sandy bottoms that are wide and flat. They exhibit extensive changes in water level and chemical content. Daily temperature changes are often of wide magnitude, sometimes ranging from 30 to 70 F in the winter, and from 70 to approximately 100 F in the summer.

2. At low winter-time temperatures the pupfish were aggregated in deep areas of quiet water. There appeared to be age and sexual differences in degrees of activity at low temperatures, with females and juveniles being more active than males.

3. Emigration from the deeper areas occurred at temperatures above 50 F. The pupfish moved from these areas in schools. In the summer they exhibited more of a tendency to remain in shallow areas, but at all seasons there was some movement to deeper areas shortly before nightfall.

4. Feeding activity was minimal at temperatures lower than 40 F. At 40 to 50 F they tended to move about in tight schools, periodically stopping to feed. Feeding intensified with increased temperatures.

At temperatures above 60 F they exhibited less tendency to move as schools and became widely dispersed over the bottom. This was associated with increased aggression in the feeding population.

5. Excluding males in breeding territories, pupfish fed primarily in aggregations of from several to hundreds of fish. They are primarily bottom-feeders but some feeding occurs at the surface and in midwater. Based on intestinal content they are omnivorous in their food habits.

6. Females fed more actively than males defending breeding territories. In both groups, feeding activity increased gradually during the day until early afternoon when it reached a plateau of maximum intensity. Feeding activity remained high until shortly before nightfall when it declined somewhat.

7. In early spring there seemed to be a general upstream migration in one area, and at all times of the year there was extensive "local movement" of the population. These movements probably serve at least three functions: (1) dispersal of breeding males, (2) increased male-female encounters, and (3) more efficient utilization of food resources.

8. Based on observations of the spawning act, the reproductive season apparently begins in February and continues into November. Spawning activity was maximal in spring and summer months. Spawning was observed at all hours of the day at temperatures ranging from 55 to 93 F. There was a shift from primarily afternoon spawning in the winter to day-long spawning at moderate spring temperatures to primarily morning spawning at summer temperatures. In the summer the frequency

of spawning reached a peak at mid-morning and then slowed until completely over at around noon when the water temperature approached 90 F. Spawning was sporadic in September, October, and early November. Late in the reproductive season the majority of the breeding population was young-of-year individuals.

9. The motor patterns of feeding, reproductive, agonistic, and maintenance behavior were described in some detail with brief comparisons with the literature on other fishes, especially other members of the Cyprinodontinae. It was noted that the spawning clasp of *C. rubrofluviatilis* differs significantly from that reported for the closely related *C. variegatus*.

10. The pupfish exhibits two functionally different types of territorial activity. These were referred to as breeding territoriality and feeding territoriality. Feeding territories were small circular areas only slightly larger in diameter than the length of the fish. They were more or less ephemerally located with the fish defending one feeding site and then moving to another. Feeding territories were defended by juveniles as well as adult males and females. Fish defending feeding territories tended to restrict their feeding to specific spots more than other members of the feeding population.

11. Breeding territories were defended by males. They were located in shallow water and usually were well-defined topographically. The territories were highly variable in size and shape, depending on a number of interrelated factors. They were roughly dome-shaped in three-deminsional aspect. In the winter, males began defending breeding

territories when the water temperature approached 55 F. Later in the season the territories were defended from dawn until dusk. At temperatures exceeding 90 to 95 F a number of males began defending feeding areas within the breeding territory. Many males, especially those in the shallowest water, deserted their territories altogether at high temperatures. In the winter, males deserted their territories at night and returned to them when the water temperature neared 55 F the next day. There was more of a tendency for males to rest at night within their territories in the summer.

12. Breeding males were very aggressive in territorial defense. Most of the agonistic encounters with neighboring males occurred at the boundaries between territories. During reproductive periods the males courted almost every intruding female. Preliminary courtship maneuvers consisted primarily of driving, looping, and zigzagging, activities that function to keep the female within the territorial boundaries of the courting male. On a few occasions females swam directly to the center of the territory as if attracted to the male for reproductive functions; however, most male-female encounters seemed incidental to the movements of the females from one place to another.

13. The relative frequencies of driving, looping, and zigzagging were variable, depending on the time of year. Seasonal changes in courtship behavior were correlated with apparent changes in female receptivity. Courtship usually ended with the female fleeing as the male attempted to contain her within the territory. A large proportion was terminated by interference from other males, demonstrating that this was a significant factor in determining the reproductive success

of individual males.

14. Two major functions were postulated for breeding territoriality, these were: (1) protection of the fertilized eggs, and (2) provision of sufficient space to allow the resident male to court females in relative freedom from interferences by conspecific males.

15. It was speculated that breeding territoriality in *Cyprinodon* may consist of the basic components of feeding territoriality superimposed upon the "primitive" type where the territory existed only with reference to the female being courted.

16. Basically there are six different color patterns in the pupfish. Certain color patterns are characteristic of sustained periods of particular activities. The color patterns and associated behavior were discussed in some detail. There was a correlation between the occurrence of one component of the color patterns, the black iris, and relative aggressiveness in the individual pupfish. Black-iris fish were more aggressive than silver-iris fish This correlation was evident in females and non-breeding males, as well as in males defending breeding territories. Spawning females exhibited a silver iris, presumably as an appeasement response that helps reduce the male's aggressiveness enough to allow successful courtship.

17. Three major species associations were found within the range of the pupfish. The *Notropis lutrensis*-*Gambusia affinis*-centrarchid association was more frequent at lower dissolved solids, while the *Hybognathus placita*-*Notropis bairdi* and *Cyprinodon rubrofluviatilis*-*Fundulus zebrinus* associations occurred more frequently at the mid-

and high-range of total dissolved solids, respectively. There was a significant drop in the number of species taken at dissolved solids higher than 12 ppt. The pupfish and _F_. _zebrinus_ were the only fishes collected at dissolved solids higher than 20 ppt.

18. The pupfish occurred in greater abundance and represented larger proportions of the ichthyofauna in regions of high dissolved solids and low species densities. This demonstrates its ability to exploit niches left vacant by the absence of other species at higher dissolved solids. Several characteristics point to its being adapted to a broad ecological niche. This probably brings the pupfish into competition with a number of species in areas of low dissolved solids where the available resources are usually partitioned among a number of more specialized species.

19. In contrast to the pupfish, the abundance of _F_. _zebrinus_ was not greatly affected by the number of species at the collection site. _F_. _zebrinus_ may be adapted to a relatively narrow ecological niche, rendering it more efficient than the pupfish in waters having low dissolved solids and supporting a complex ichthyofauna.

20. The abundance of _F_. _zebrinus_ varied negatively with the dissolved solids content of the water. Evidence was presented which demonstrated that there may be a high level of competition with Red River pupfish at higher dissolved solids. Competition for food and spawning sites, added to the generally higher aggression of pupfish may account for the observed depression of the _zebrinus_ population at higher dissolved solids.

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