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Changing Preference for Substrate Color by Reproductively Active Mosquitofish, *Gambusia affinis* (Baird and Girard) (Poeciliidae, Atheriniformes)

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INTRODUCTION

The intimate relationship between the color pattern of an animal and its environment is generally considered to be a fundamental ecological determinant. The entire mode of life, including the avoidance of predators or the capturing of prey, food preferences, temperature adaptation, reproduction, and the care of young, is strongly influenced by this relationship.

The adjustment of color pattern of a species to a part of its habitat, in some instances, may be a permanent one built up over a long expanse of evolutionary time; in other instances, a mechanism for color and pattern change that permits a variable individual adjustment to more than one niche may be developed. Especially among the lower vertebrates, individual responses to local conditions are widespread; the response of the pigmentary system may be physiologically or neurologically controlled and may manifest itself over a period of days, hours, minutes, or seconds. Among the lower vertebrates, fishes show by far the

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greatest diversity of mechanisms for color change, although very few of the species with such a capability have been studied critically and with full regard to the many interrelated aspects of color and pattern adaptation in nature. Moreover, most past studies of color adaptation in fishes have dealt with species that possess unusual and sometimes bizarre capabilities for rapid color change under direct nervous control. The work on the gradual, physiologically induced, color change in the mosquitofish, *Gambusia affinis* and related fishes by Francis B. Sumner, forms an outstanding exception to that generalization. In a series of papers, Sumner (1934a, 1934b, 1935a, 1935b, 1940a, 1940b), and Sumner and Doudoroff (1937, 1938) studied the relationship of physiologically induced color change to environmental background shade and correlated the pigmentary adaptation produced by exposure to the substratum with predation. As a by-product of their researches, Sumner and Doudoroff (1938) deduced a relationship in *Gambusia* between a physiological, and the consequent pigmentary, response to background shade and a susceptibility to infectious disease-producing agents in the laboratory environment. The sensitivity of response of *Gambusia* to substrate color established by Sumner's work, notably the ability of the fish to harmonize with the substratum and its significantly greater susceptibility to disease when forced to remain over predominantly dark backgrounds, became of especial interest to us in connection with a long-term study of the pond ecology of *Gambusia affinis* conducted at the Kalbfleisch Field Research Station of the American Museum of Natural History at Huntington, Long Island.

During the four-year period of the Long Island project, we had repeatedly noted not only that fish which are more or less stationary over light sand bottom are lighter in color than fish over dark mud bottom, but also that the predominance during midday of either light-phase or dark-phase fish in the pond fluctuates in a periodic manner during the late spring and summer months. The onset of the periodicity in midday color-phase, and the concomitant periodicity in substrate selection (sand or mud), was found to correspond with the onset of the reproductive season. The discovery of that relationship prompted an investigation, reported below, of the reproductive cycle and habitat selection in our northern-hardy strain of *Gambusia affinis.*

Of all of the more than 150 species of the viviparous fish family Poeciliidae, *Gambusia affinis* has the most extensive and ecologically diversified natural range. It occurs from the southern tip of New Jersey southward along the Atlantic coastal plain, throughout peninsular Florida, along the Gulf coastal plain, and northward in the Mississippi

Valley to southern Illinois and Indiana, and south in Mexico to the Río Pánuco Basin of northern Veracruz (Rosen and Bailey, 1963). Its latitudinal range, from the temperate zone to the lowland tropics, is matched by its broad tolerances for fresh- and brackish-water environments which are often of a most inhospitable or ephemeral kind. Its wide spectrum of tolerances has greatly contributed to its usefulness in mosquito abatement programs in innumerable countries around the globe (Rosen, 1957; Myers, 1965). On the basis of its extensive natural range, its capability for colonizing diverse habitats, its great reproduction rate and sometimes astounding local abundance (see Hildebrand, 1918, and below), *Gambusia affinis* may justifiably be said to be one of the outstandingly successful freshwater fish species of the world. Clearly, a knowledge of the ecology of this species is essential for an understanding of its success in nature.

Acknowledgments

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MATERIALS AND METHODS

The Environment: The permanent pond at the Kalbfleisch Field Research Station is situated in a depression at the base of a hill. It is roughly rectangular and measures 70 by 80 feet when filled to a normal early summer depth of 3 feet in the deepest area. The northern grassy shore slopes steeply into sandy shallows, whereas the eastern shore of grass, trees, and shrubs falls off more gradually to form a broad, uniformly shallow area of mud bottom. The southern and western shores are bounded by a vertical stone wall; the western side of the pond, inside the wall, is marshy.

With normal rainfall, the maximum depth of the pond varies from 3 to 4 feet in spring to about 2 feet in late summer and early autumn (fig. 1). Depth is measured by a gauge that is situated in the south about 8 feet from the wall in the deepest part of the pond. During the summer the northeastern one-third of the pond has a depth of less than

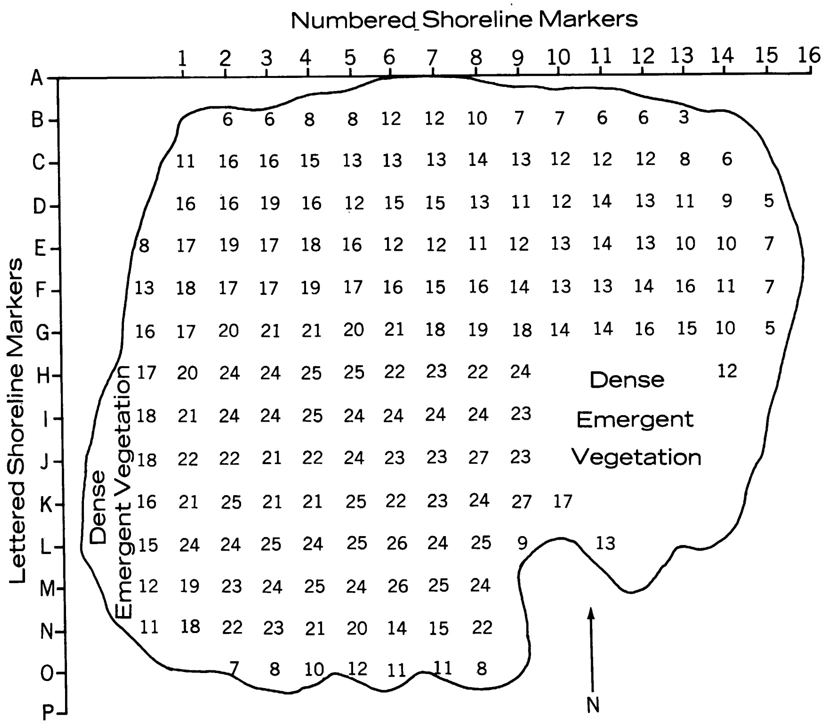

FIG. 1. Bathymetric chart of Kalbfleisch Pond showing depth variation in inches when maximum water depth is 27 inches at points J8 and K9. All lettered and numbered marker stakes at 5-foot intervals.

1 foot. During 1964 and especially in 1965, a serious drought, which affected much of the northeastern United States, resulted in a drop in water level to a gauge depth of about 1 foot. Two-fifths of the eastern end of the pond was completely dry, and the average depth of the remainder of the pond was less than 8 inches. In order to bring the depth up to its former level, 160,000 gallons of well water were added during the summers of 1964 and 1965. This addition served to maintain a constant gauge depth of 3 feet during the observation periods.

A continuously changing shade pattern is cast over the water surface by trees and a building near the pond (fig. 2). This pattern varied only slightly from year to year as a result of vegetation growth and fluctuations of the pond boundaries during the drought.

Pond temperatures ranged from a mean February high of 4.0° C. recorded in the deep areas, to a mean July high of 32.2° C. recorded

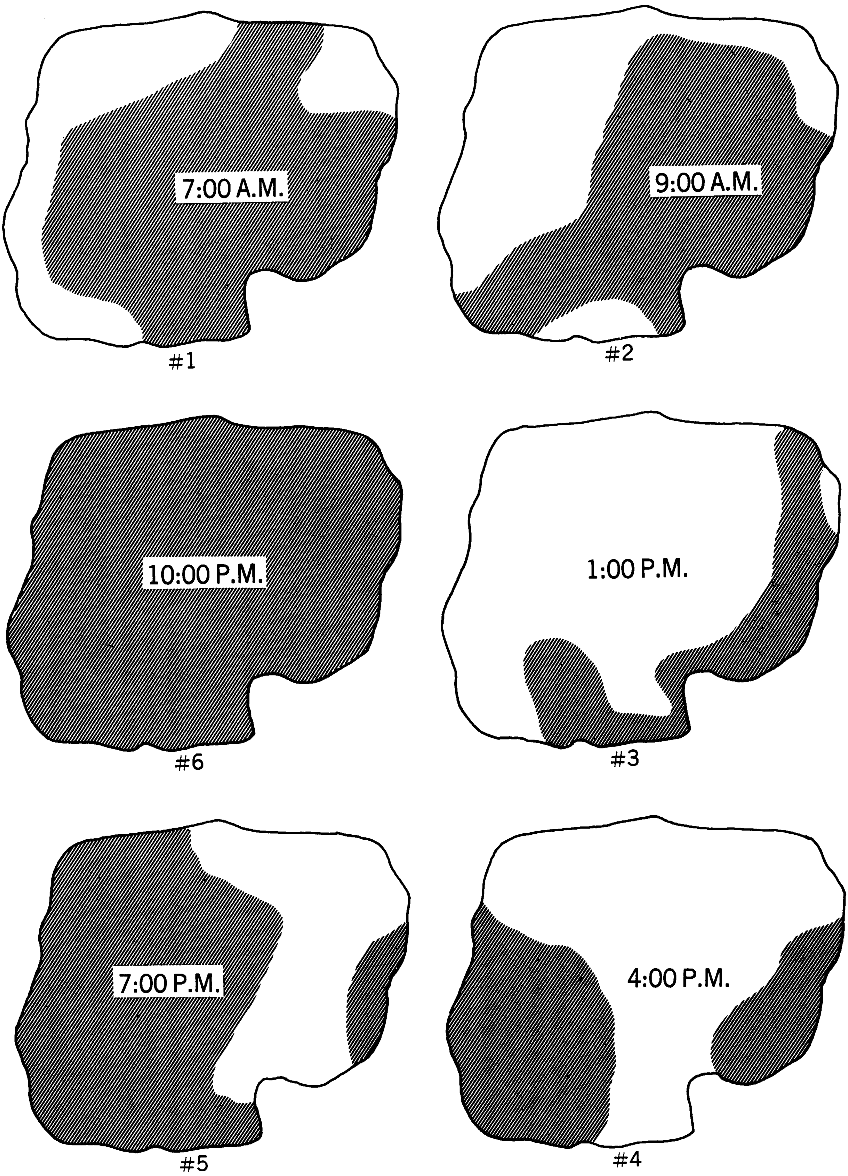

FIG. 2. Sun and shade pattern on Kalbfleisch Pond for June 23, 1965. Shaded areas are crosshatched.

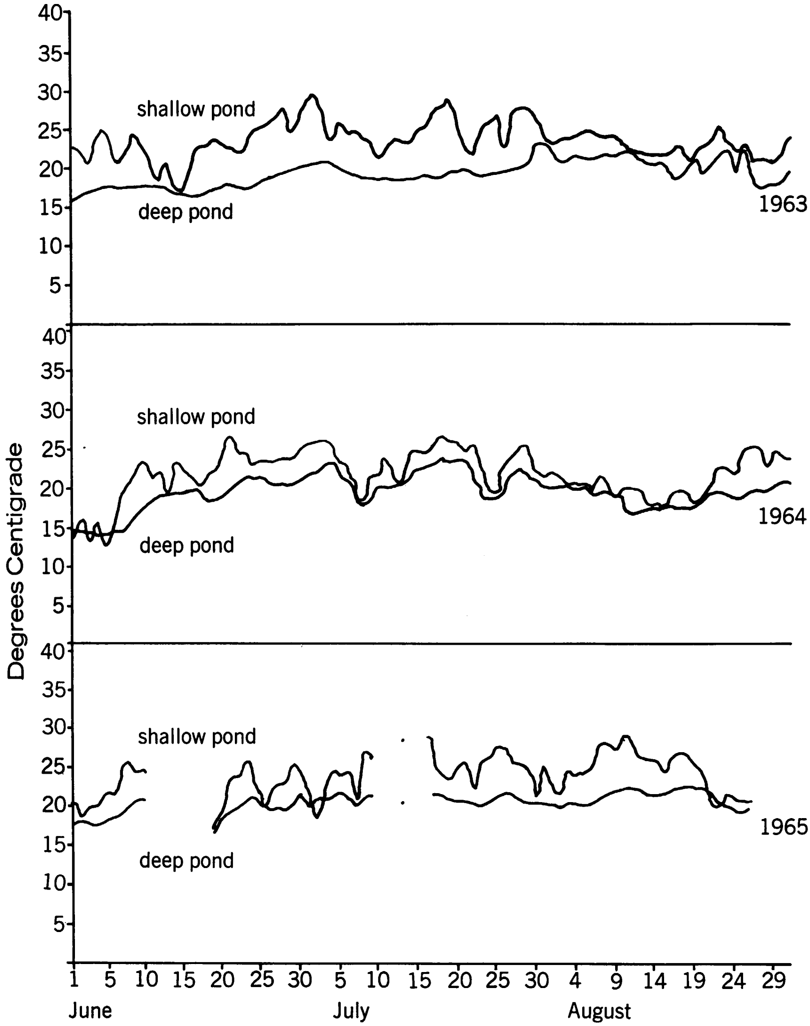

FIG. 3. Median shallow and deep-pond temperatures of Kalbfleisch Pond for the three summers of the study period. Breaks in the lines for the 1965 temperature graph represent malfunctions of the recording instrument.

at the surface. A Honeywell thermocouple recorder maintains a constant record of shallow and deep pond and of ambient temperatures throughout the year (figs. 3, 4).

The water in the pond is clear, and has a faint brownish green color.

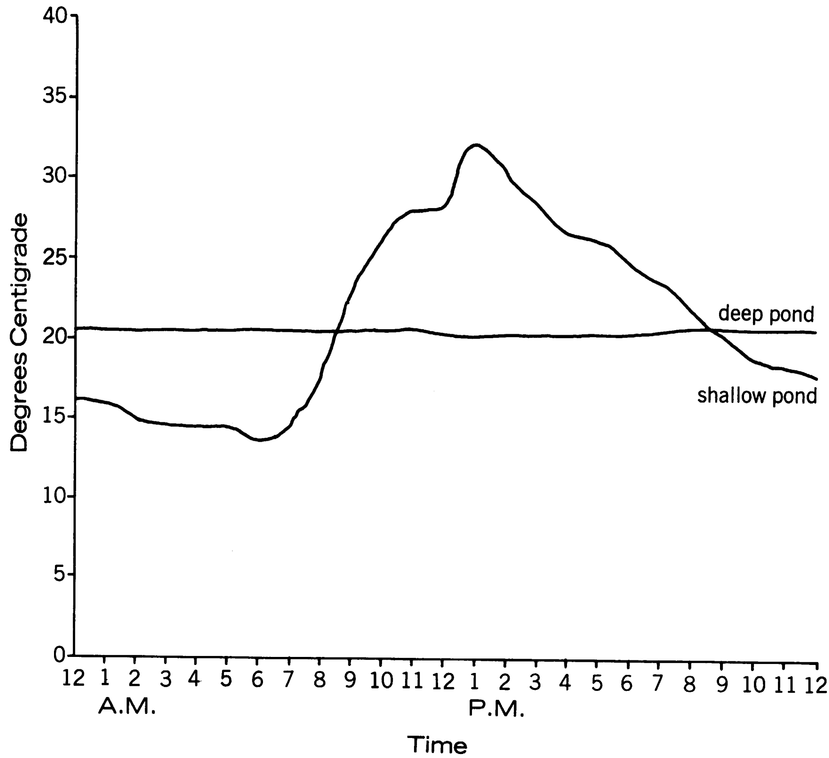

FIG. 4. Typical daily summer shallow and deep-pond temperature fluctuations at Kalbfleisch Pond (for July 30, 1965).

The pH averages 7.2.

The pond supports a large invertebrate fauna. Table 1 lists the resident invertebrates recorded in 1964 and 1965. These consist of zooplankton and phytoplankton, gastropods, ostracods, and coelenterates. Transient invertebrates include various insect larvae, such as mosquito, midge, and dragonfly larvae.

Non-piscine aquatic vertebrates include the painted turtle (*Chrysemys picta*) and two resident species of anuran (*Rana catesbeiana, R. clamitans*).

There are six major species of plants in the pond. Their distribution in early June is shown in figure 5. Two of these plants, *Poa* and *Polygonum,* spread over a considerable area during the summer and must be managed to prevent their spreading over the entire pond. The plants are uprooted and removed until the pond is restored to the early June condition. Figure 6 shows the pond just before management.

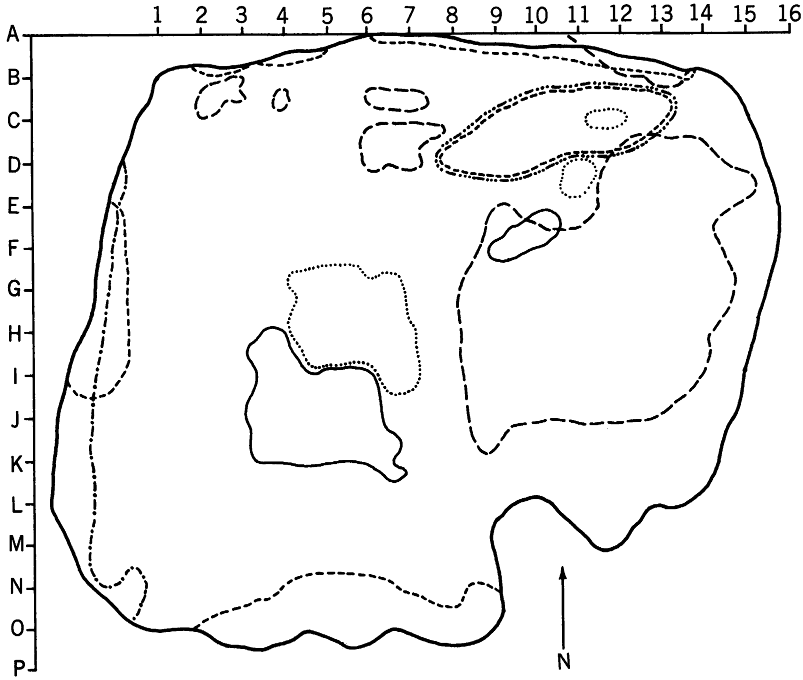

FIG. 5. Kalbfleisch Pond vegetation communities. Distribution given for June 21, 1965. Solid line, *Nupha variegatum;* small dashes, *Polygonum hydropiperoides;* large dashes, *Typha angostifolia;* dash and dot, *Phragmites communis;* dotted line, *Potamageton natans;* dash and three dots, *Poa* sp.

In addition to differences in water depth, the pond offers a variety of aquatic habitats. The south and west have a bottom of brown mud, whereas white sand forms the substrate along the northern shore. Cover is provided by a dense stand of *Phragmites communis* along the west shore in shallow water, and a stand of *Typha angostifolia* in deeper water.

THE FISH POPULATION: In August, 1961, 10 female and 12 male *Gambusia* of a cold-hardy strain were introduced in the Kalbfleisch pond. These fish derived from a strain originated in Chicago in 1937 by L. A. Krumholz. The progeny resulting from this introduction formed the basis of the observations from 1962 through 1964. An abnormal winter-kill in 1964–1965 resulted in the complete loss of the population in the permanent pond. In May, 1965, 12 females and 14 males were introduced into the pond from a reserve stock. All of the 1965 studies were based on the progeny of that restocking.

TABLE 1
INVERTEBRATE FAUNA OF KALBFLEISCH POND BASED ON SAMPLES TAKEN DURING THE SUMMERS OF 1964 AND 1965

Class	Order	Family	Genus
Mastigophora	Chrysomonadina	Ochromonadidae	*Uroglena*
		Syncryptidae	*Synura*
	Cryptomonadina	Cryptomonadidae	*Chilomonas*
	Dinoflagellata	Gystodiniidae	*Glenodinium*
		Peridiniidae	*Peridinium*
	Euglenoidina	Euglenidae	*Euglena*
	Phytomonadina	Volvocidae	*Volvox*
Ciliata	Holotricha	Colepidae	*Coleps*
		Parameciidae	*Paramecium*
	Spirotricha	Halteriidae	*Haltera*
		Oxytrichidae	*Oxytricha*
		Spirostomiidae	*Spirostomium*
		Stenoridae	*Stentor*
Hydrozoa	Hydroida	Hydridae	*Hydra*
Oligochaeta	Plesiopora	Aeolosomatidae	—[a]
Gastropoda	Pulmonata	Ancylidae	*Ferrissia*
		Lymnaeidae	—
		Planorbidae	—
Crustacea	Cladocera	Chydoridae	*Alona*
		Daphnidae	*Daphnia*
			Simocephalus
	Eucopepoda	Cyclopidae	*Cyclops*
		Diaptomidae	*Diaptomus*
	Podocopa (Ostracoda)	Cypridae	*Cypridopsis*
Insecta	Coleoptera	Dytiscidae	—
		Elmidae	—
		Gyrinidae	—
		Halipidae	—
		Hydrophilidae	—
	Diptera	Ceratopogonidae	—
		Culicidae	*Chaoborus*
		Tendipedidae	—
	Ephemeroptera	Baetidae	—
	Hemiptera	Notonectidae	—
		Corixidae	—
	Odonata	Aeshnidae	*Anax*
		Coenagrionidae	—
	Plecoptera	—[b]	—[b]

[a] Identification to family only.
[b] Identification to order only.

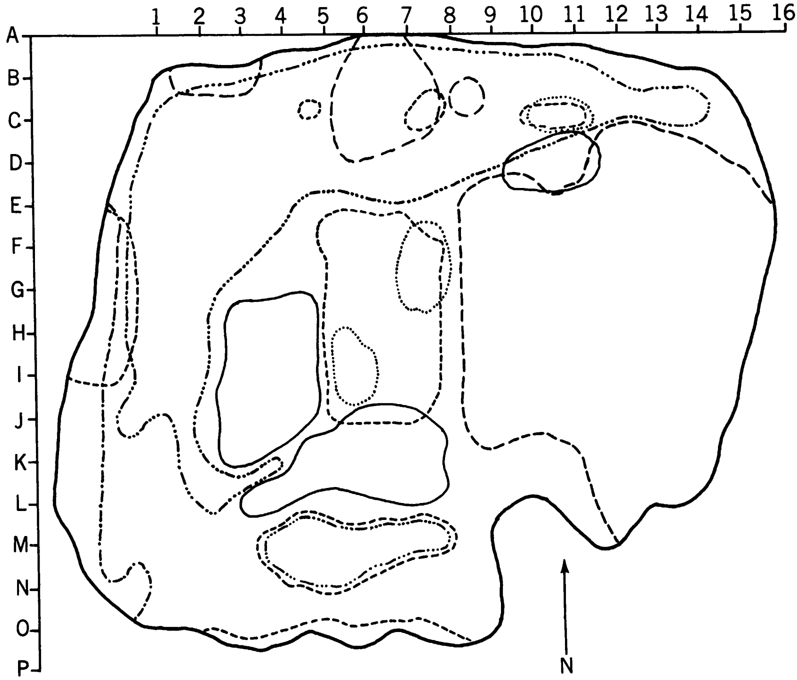

FIG. 6. Kalbfleisch Pond vegetation communities. Distribution given for July 15, 1965 just before management. Symbols as in figure 5.

To facilitate observation of fish distribution a grid system was established by setting numbered and lettered posts 5 feet apart, along the western and northern shores (fig. 1). The pond was thereby divided into approximately 200 imaginary square sectors 5 feet on a side. The distribution of fish was recorded from June to September in 1963, 1964, and 1965. The number of males and females in each sector and the approximate position of immature fish were recorded on a map of the pond. Observations were made daily at 7 A.M. and 9 A.M., and 1 P.M., 4 P.M., 7 P.M., and 10 P.M. Fish were observed from the shore and from a boat. At night a high beam flashlight was used to observe fish distribution. At all times all fish seen were recorded.

In this pond the major predators of *Gambusia* include the belted kingfisher (*Megaceryle alcyon*), the green heron (*Butorides virescens*), water boatmen (Corixidae), Odonata nymphs (*Anax* sp.), the common water snake (*Natrix sipedon*), and the garter snake (*Thamnophis sirtalis*). Of these, the kingfisher and heron account for the major loss of adult fish due to predation, and have been observed taking fish on a number of occasions. The corixids have been seen attacking young fish, and the Odonata nymphs, adult fish.

The total seasonal reproductive period of the population, as deduced from the appearance of newborn young, extends from May to late September or early October. Young appear in waves beginning in late May or early June and every 3 to 4 weeks thereafter until late in September. The first waves are well defined; the adult females drop their young at about the same time. By late August, however, these females are out of phase with one another. Also, at this time, first-wave fish are in their first reproductive phase so that individual waves are difficult to delimit. In 1963 distinct waves of young appeared in late May, on June 13, July 14, and August 17. During 1964, the dates of appearance of young were late May, June 10, June 27, and August 5. In each case young appeared during a period of about a week. Second-year adults were observed to have four or five broods during a reproductive season. Young-of-the-year females produce no more than two broods by September of the same year.

In June the sex ratio is about 1:1 but by September the females outnumber the males by as much as 10:1.

RESULTS

Diurnal Movements and General Distribution Patterns

During the summer of 1963, scheduled observations were made five times daily from late June to early September revealing a fairly regular pattern of movement of the fish, from deeper areas of the pond in the south to the shallow northern shore. The inshore daily migration of the adult population was in general clockwise, going from the south along the western marshy boundary of the pond until the northern shore was attained. Thereafter, the fish gradually dispersed eastward until a major part of the northern shore was occupied by small-sized to moderate-sized groups composed principally of adult females.

In 1963 it was also noted that in the early part of the breeding season, in late May and early June, males and females tended to move together, the males fighting among themselves or pursuing females. Apparently because of the intense sexual activity, the early seasonal movements of the adult population were somewhat disrupted and the precise diurnal migration patterns of the summer months were not yet evident. Presumably by the onset of summer (that is, late June), the bulk of the overwintered females had been inseminated, and the males and females

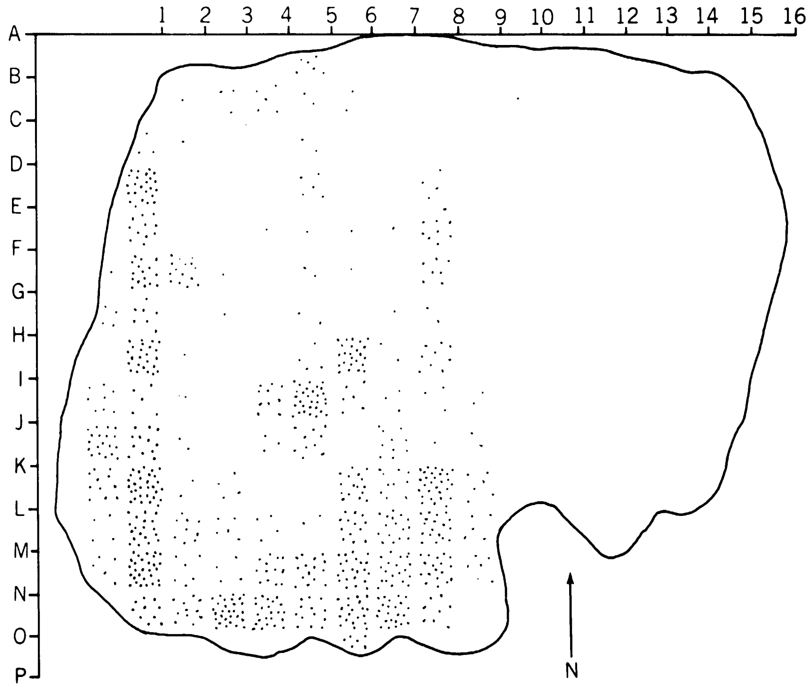

FIG. 7. Twenty-four-day composite distribution pattern for adult *Gambusia* in Kalbfleisch Pond at 07:00. In this and the following five figures (8–12), each dot represents one individual.

then moved about in more or less separate groups. The pattern of movement of the population was, from that point onward into the reproductive season, based largely on the movements of adult females, which were always in far greater abundance. Small groups of males could be seen scattered here and there among the females, although males would also be seen elsewhere in the pond away from the main concentration of fish.

Observations made in the cooler months of 1962 through 1964 before breeding had begun (March and April) and after it had ceased (November and December) revealed similar daily migratory movements, although fewer fish were in evidence at these times. During January and February, the pond was periodically covered with ice and little or no fish activity was apparent.

In 1964 and 1965 observations were made continuously throughout the warm weather and the actual number of fish were recorded in each sector of the pond during the five regular daily observation periods and,

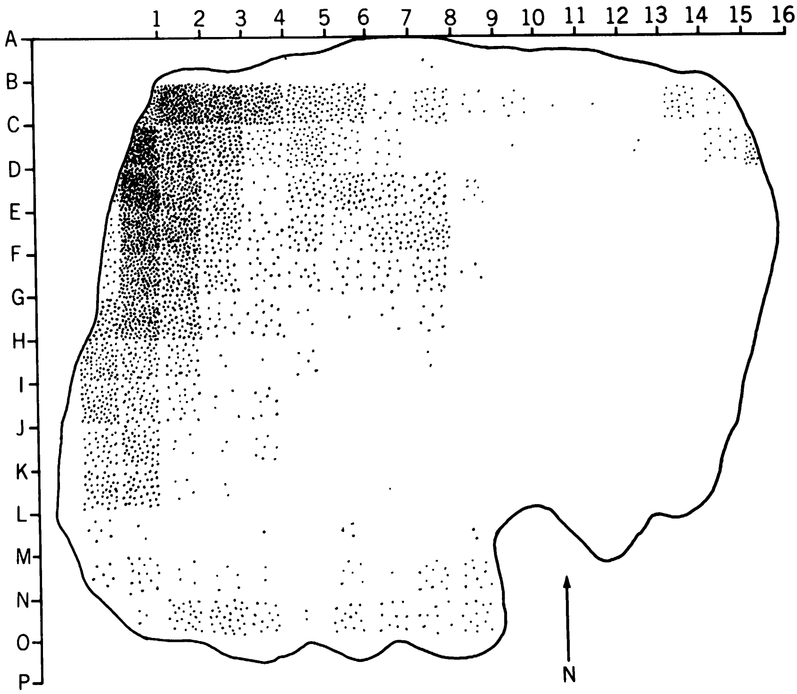

FIG. 8. Twenty-four-day composite distribution pattern for adult *Gambusia* in Kalbfleisch Pond at 09:00.

in 1965, during a night observation at 10 P.M. A few late night observations were also made in the 1964 season.

Relatively few fish were recorded during the early morning observations, but the number recorded increased until midday when the largest numbers of individuals were seen. Presumably, fish moved from the deep areas to join the observable concentration on the surface as the day progressed, resulting in the increase in recorded individuals in midafternoon. Composite distribution maps for the same five observation times and for a 10 P.M. observation for 1964 are shown in figures 7–12. This diurnal pattern was observed with only minor variations from 1963 to 1965.

In the early morning soon after sunrise, fish could be seen near the surface and in deeper water in the south. As the day progressed, the major concentration of adult fish moved along the western and northern shores and were concentrated in the east shortly before sunset. Nighttime observations showed that the fish continued their migration southward and westward, and by 10 P.M. they were once again in deep water. Most

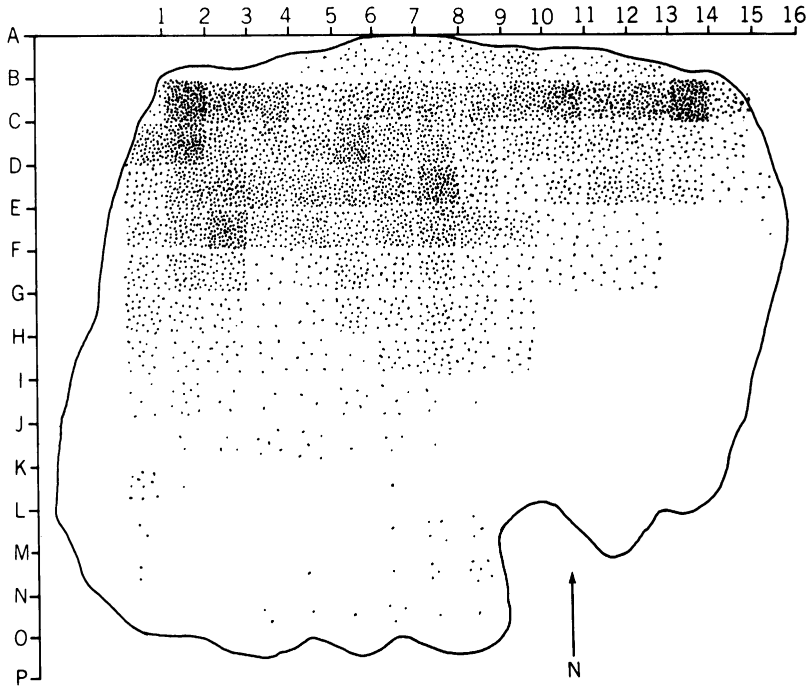

FIG. 9. Twenty-four-day composite distribution pattern for adult *Gambusia* in Kalbfleisch Pond at 13:00.

fish at this time were seen below the surface near the bottom. In 1964 the night observations showed that the fish were distributed over a wide area of the pond, but they were always near the bottom and in areas of moderate to deep water. In 1965 the 10 P.M. distribution was the same as the early morning one, fish being seen only in the deep area of the south.

During 1965, a serious drought reduced the pond surface area by half. Although the pond was much abbreviated, the typical diurnal pattern of migration persisted; the fish moved clockwise along the new shoreline during the day and into the south at night.

When both open water and cover were available in the areas through which fish moved during their daily migrations, individuals were seen to dart in and out of the vegetation forming the cover. When only open water was available, the slightest disturbance on shore resulted in the rapid movement of fish into distant vegetation or into deeper water. After several minutes they would return slowly to their former position.

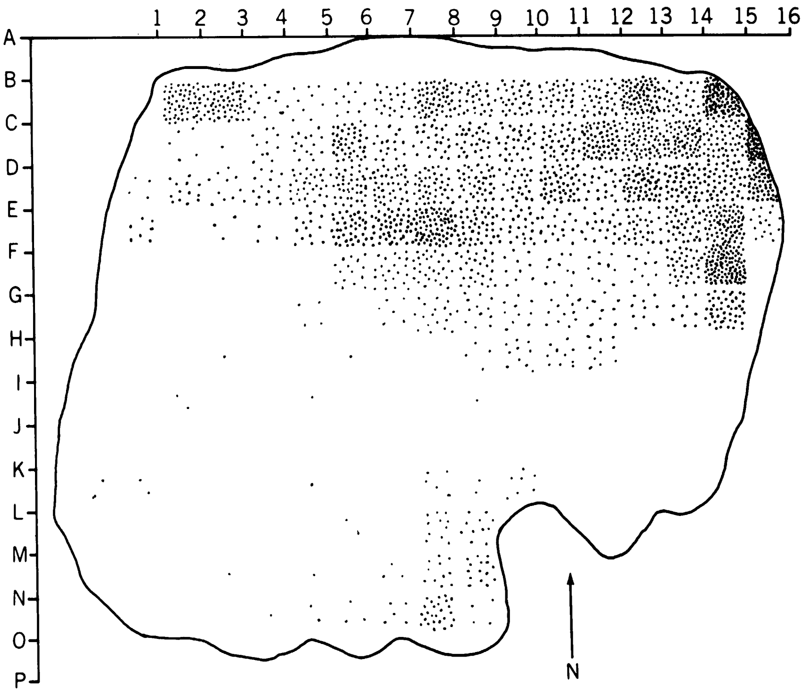

FIG. 10. Twenty-four-day composite distribution pattern for adult *Gambusia* in Kalbfleisch Pond at 16:00.

The distribution of immature fish differed from that of the adults. From birth to the age of about one week fish remained in the area in which they were born. During the night the young fish simply settled to the bottom and remained there until sunrise. Fish between two and three weeks old moved into deeper water at night and returned to the shallow areas during the day. Their distribution was more extensive than that of the adults, occurring over larger areas of the pond at any given time. At about four weeks of age, young fish began to move with the adults through the normal daily pattern.

An important feature of the daily clockwise movements of adults is the spatial separation along the light-bottomed, sandy northern shore, of females with and without advanced embryos. Gravid females (i.e., those nearly ready to deliver a brood and distinguishable by their robust outline and large, dark periproctal spot) were concentrated near shore directly over light sandy bottom. Non-gravid females or those in early pregnancy, which have a slender outline and insignificant peri-

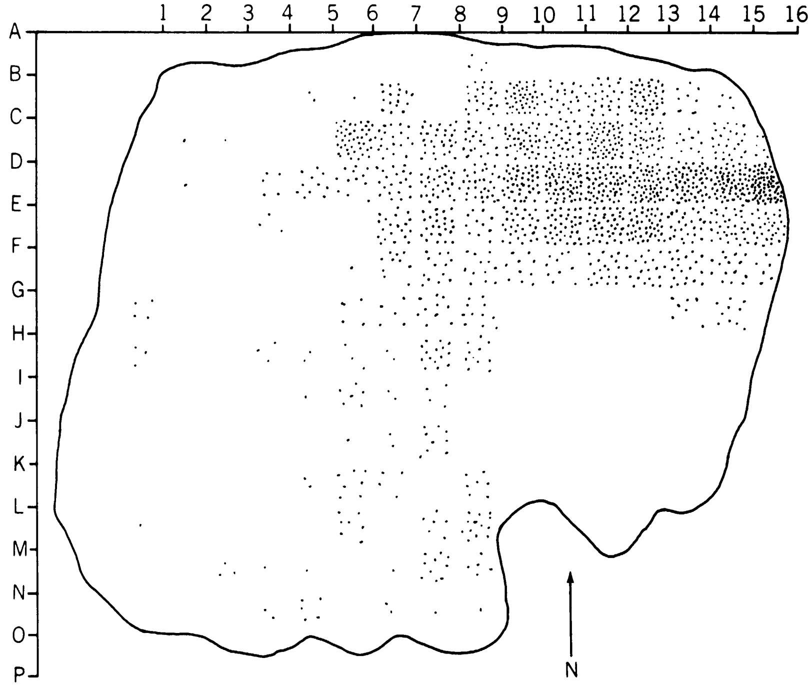

FIG. 11. Twenty-four-day composite distribution pattern for adult *Gambusia* in Kalbfleisch Pond at 19:00.

proctal pigment, were concentrated about 1 to 3 feet offshore on the edge of the sandy slope or over dark mud bottom. As females from the same year class had roughly synchronous breeding cycles (one brood approximately every 24 to 30 days), the majority of females early in the reproductive season would be either directly over sandy bottom or farther offshore at any given time. As the season advanced, brood synchrony became less and less evident and the simultaneous spatial separation of gravid and non-gravid females became more evident along the northern shoreline. These observations prompted a laboratory investigation of substrate preferences in reproductively active females, the results of which are presented below.

POND TEMPERATURE DISTRIBUTION: Throughout the three-year period of the detailed observations, the most striking behavioral characteristic of *Gambusia* in the Kalbfleisch pond was the diurnal movement pattern discussed above. The position of the major concentration of fish at any time during the day roughly corresponded to those areas of the pond

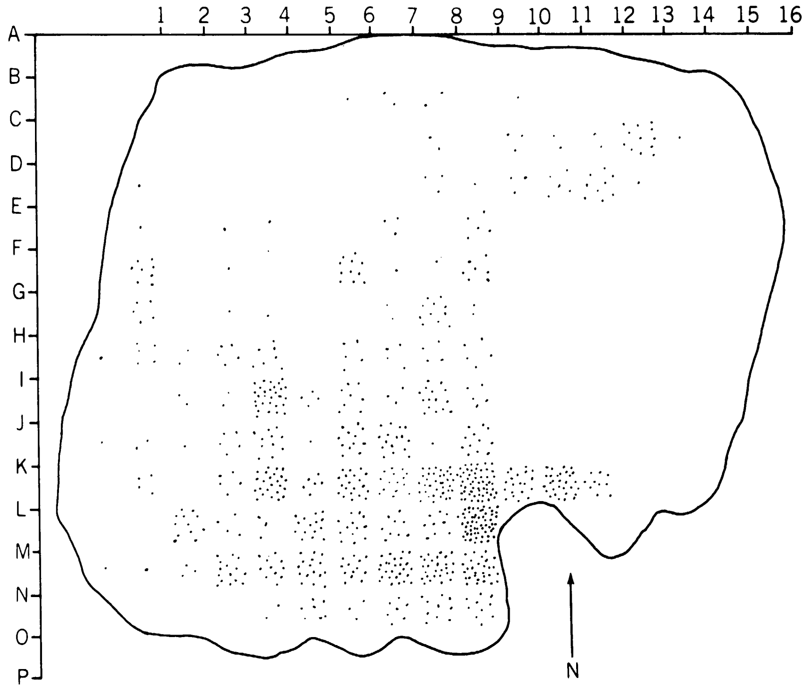

FIG. 12. Twenty-four-day composite distribution pattern for adult *Gambusia* in Kalbfleisch Pond at 22:00.

receiving direct sunlight. It seemed plausible that the fish were responding to light or to some other factor directly related to light, such as water temperature or the movement of food organisms. The extension of a daily pattern of movement into the evening, and the presence of an entire diurnal cycle on overcast days, indicated that light is not necessarily the only factor that induced the diurnal migratory activity.

It was also noted that during the night, in the early morning, and late evening fish were not active on the surface, but remained in deeper water where the temperature was several degrees higher. When surface temperatures exceeded 35° C. in the midafternoon, fewer fish were observed on the surface. At such times more fish were seen below the surface where the temperature was several degrees lower.

On several days in 1964, surface temperatures were recorded in various areas of the pond to determine the pattern change during the day. The highest recorded temperatures, in each case, were found to occur in successive areas that corresponded to the clockwise movement of the fish and sunlight pattern around the pond.

All of the foregoing observations led us to suspect that the observed cyclic movements of the fish were influenced mainly by water temperatures. The behavior of the fish appeared to be in direct response to water temperatures. The presence of direct sunlight merely accentuated normal temperature differentials around the pond. More detailed temperature studies were conducted in 1965 to examine the relationship between temperature and fish distributions. The 1965 data, with temperature records and observations made over the entire three-summer period, support our initial hypothesis of diurnal fish migrations induced by temperature. The accumulated evidence is summarized in the following paragraphs.

General pond temperatures vary from a mean February high of 4.0° C., which occurs in the deep areas, to a mean July high of 32.2° C., recorded at the surface. At any time during the day surface temperatures depend on the water depth and on the sun and shade pattern. Shallow areas receiving direct sunlight are warmer than shallow areas in the shade, and both are warmer than surface water over deep areas.

As mentioned above, the pattern of sun and shade changes during the day. In the early morning the southern third of the pond receives direct sunlight first and the surface rapidly warms to a temperature above that of the deeper water. As the day advances and the direct sunlight falls on shallow water, the shallows of the western, then the northern shores show the highest temperatures in the pond. The eastern corner of the pond receives the last sunlight of the day. After sunset, surface temperatures fall rapidly so that by 10 P.M. the warmest water is found in the deepest areas of the pond. The deep areas maintain stable temperatures during the night, and by 5 A.M. they average 5° C. higher than the surface temperature.

On overcast days a similar pattern of temperature change is found, but all temperatures average lower than those on sunny days and there is less temperature differential from one area of the pond to another.

Records made in 1965 of water temperature, taken one-half inch below the surface in each quadrant of the pond during the regular observations of fish distribution, provided data for studying the relationship of temperature distribution with position of major fish concentrations. Isotherms were drawn on the temperature distribution maps prepared for each of the five observation periods on each of five days in 1965. The plotted isotherms showed that the highest recorded surface temperatures occurred in adjoining areas of the pond successively and that the high temperatures progressed in a clockwise direction around the pond cor-

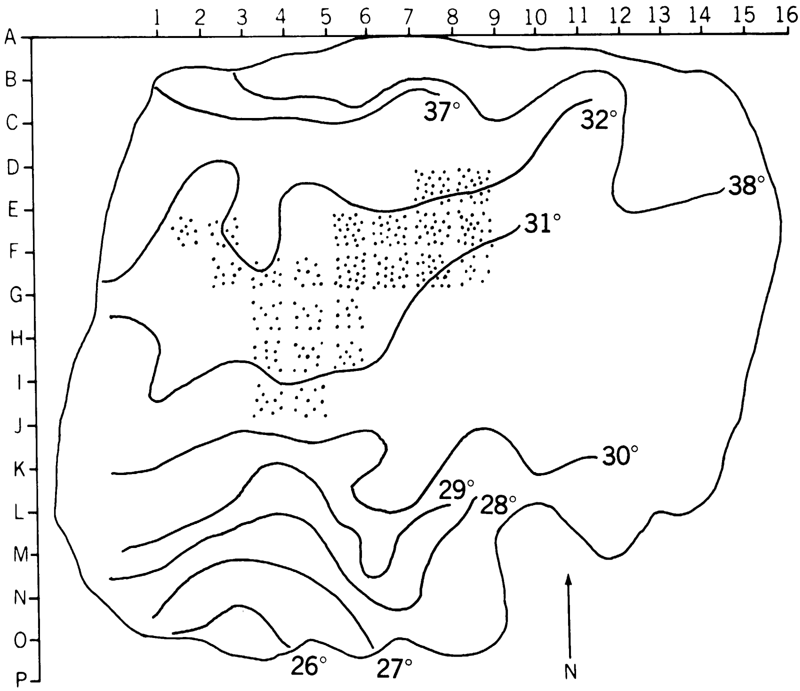

FIG. 13. Distribution pattern for adult *Gambusia* in relation to isotherms during the 13:00 observation period on July 17, 1965. In this and the following three figures (14–16), each dot represents one individual. Temperatures were recorded just below the water surface.

responding to the daily progression of direct sunlight. During mid-morning, isotherms were oriented along the western shore. In the early afternoon, they were oriented along the northern shore, and by late afternoon, they were parallel to the eastern border of the pond. The highest temperatures were nearest shore at all times.

In figures 13–16 temperature and fish distributions for the same observation periods are shown combined. Such distribution maps reveal that the major concentrations of fish occurred in the warmest water available in the pond, except when surface temperatures exceeded 33° C. The correlation appears to hold even when the isotherms are orientated in various ways with respect to the shoreline. On several occasions, near-shore temperatures were as high as 38° C., but the fish were concentrated nevertheless between the 30° and 33° C. isotherm lines. During the night when surface temperatures fell below those of the deep areas,

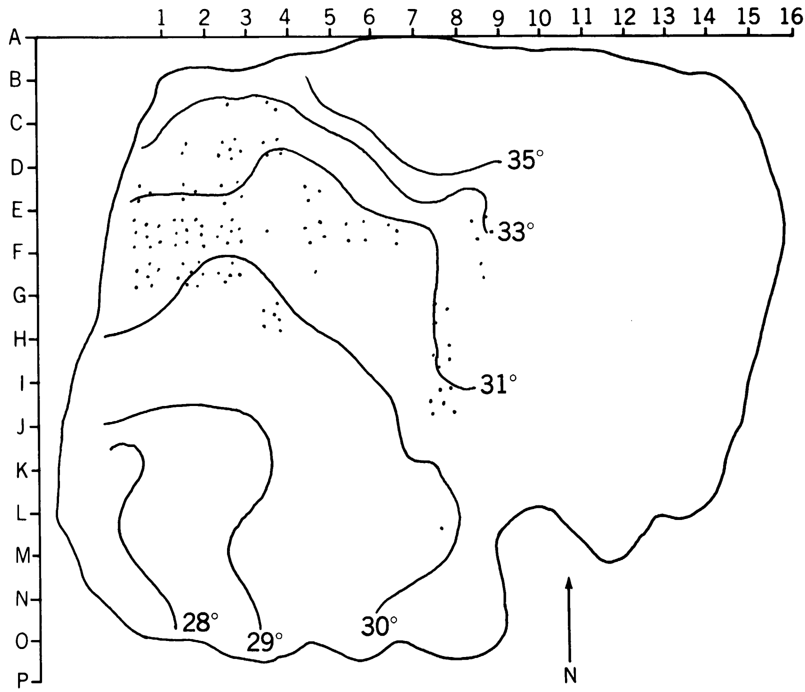

FIG. 14. Distribution pattern for adult *Gambusia* in relation to isotherms during the 16:00 observation period on July 7, 1965.

few fish were observed in the cooler, shallow water; the largest concentrations were invariably found near the bottom in the deeper areas of the pond.

FEEDING PATTERNS: The observations, cited above, on the relationship between the diurnal movements of fish and changes in surface temperatures in the pond suggest that the fish are responding thermotactically. It occurred to us that a significant part of the invertebrate fauna of the pond might show a similar response to temperature and that fish movements may be closely tied to the pursuit of food organisms. A study was therefore conducted of the distribution of the principal components of the invertebrate pond fauna and the feeding habits of the fish at different times of the day in different parts of the pond.

Table 1 lists the principal invertebrate fauna of the pond. Table 2 shows the frequency distribution of selected organisms with reference to depth of the sampling area. At least 81 per cent of the *Cyclops, Simocephalus, Alona,* Tendipedidae, and Ostracoda were found to be in the

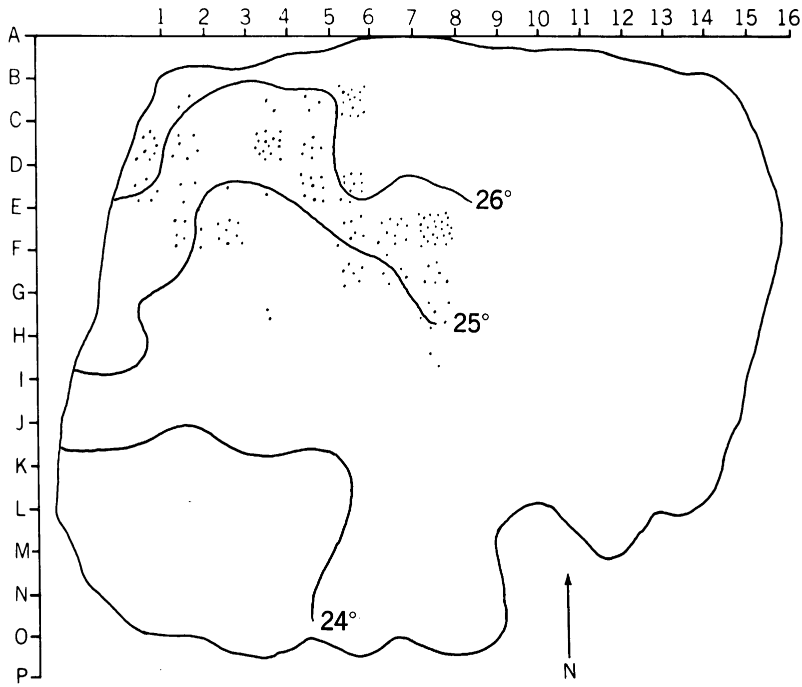

FIG. 15. Distribution pattern for adult *Gambusia* in relation to isotherms during the 13:00 observation period on August 9, 1965.

shallow water (to 8 inches in depth). In contrast, only 30 per cent of the *Diaptomus* and *Daphnia* were found in the shallow areas. It is thus possible to separate the organisms into those found along the shallow

TABLE 2

DEEP AND SHALLOW DISTRIBUTION OF MAJOR FOOD ORGANISMS

(Samples included contain 100 or more individuals per cubic foot of water)

Organism	No. of Deep Samples Containing Organisms	No. of Shallow Samples Containing Organisms	Percentage of Shallow Preference
Ostracoda	2	25	94.6
Cyclops	5	69	93.4
Alona	4	39	90.7
Simocephalus	8	68	89.5
Tendipedidae	3	13	81.3
Diaptomus	52	26	33.3
Daphnia	55	20	26.7

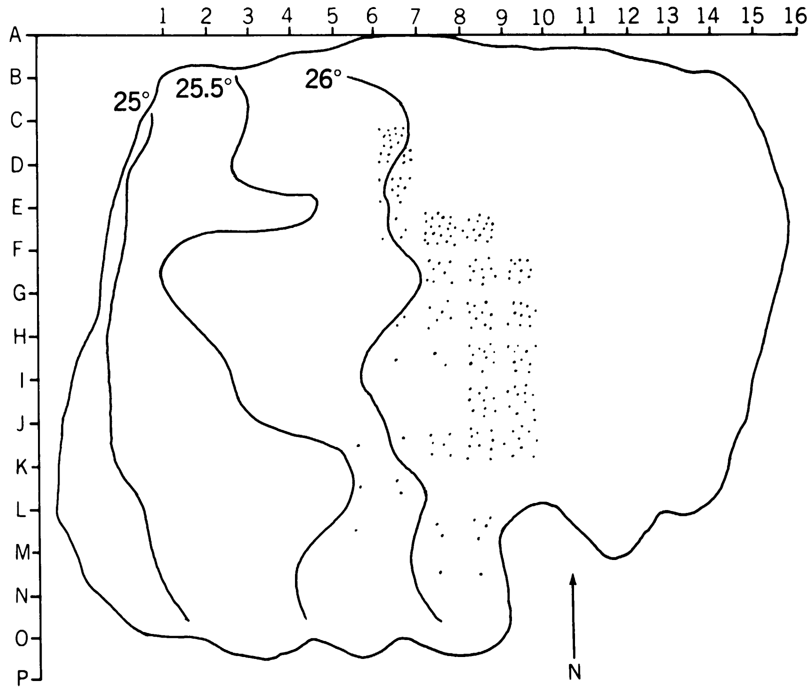

FIG. 16. Distribution pattern for adult *Gambusia* in relation to isotherms during the 19:00 observation period on August 6, 1965.

northern and western shores and those found in the deep water (8 to 24 inches in depth) areas of the south.

The daily abundance patterns for five major food organisms were compiled using daily abundance values, broken down further according to sampling area and time. In this way, it was possible to determine whether the organisms themselves moved around the pond. The abundance of *Daphnia* and *Diaptomus* was found to change slightly within the central and southern areas of the pond, roughly following changes in the sun and shade pattern. These deep areas are occupied by fish only at night. *Simocephalus, Cyclops, Alona,* and the ostracods moved in various ways in the shallow northern sectors. *Simocephalus* showed a counterclockwise trend across the north, thus moving counter to the movement of the fishes. The majority of *Cyclops* and *Alona* moved from the western shore into the shallow northern sector of the pond, although maintaining a fair abundance in the other areas. These movements also appeared to be closely correlated with changes in the sun and shade pattern. The ostracods were not present in sufficiently large numbers for their movements to be mapped. Tendipedidae were found to maintain a fairly wide distribution throughout the north. Both these and *Simocephalus* were generally found on the bottom or clinging to vegetation.

In 1964, gut content analyses revealed differences in the food organisms eaten in relation to whether the fish were in the sun or the shade. The fish in the sun ate primarily tendipedids whereas those in the shade

TABLE 3

STOMACH CONTENT ANALYSES OF *Gambusia affinis*[a]

(Number of stomachs containing organism[b] and percentage of total number of stomachs analyzed)

Contents	Day Number	Day Per Cent	Night Number	Night Per Cent
Alona	73	68.9	42	50.6
Simocephalus	58	54.7	36	43.4
Cyclops	47	44.3	13	15.7
Insecta	38	35.8	18	21.7
Tendipedidae	36	34.0	35	42.2
Diaptomus	33	31.1	11	13.3
Daphnia	20	18.9	32	38.6
Ostracoda	13	12.3	2	2.4
Odonata	3	2.8	2	2.4
Corixidae	2	1.9	3	3.6
Gambusia	1	0.9	—	—
Total No. of stomachs	106		83	

[a] For the summer of 1965.

[b] The number of stomachs containing any particular organism was based on the occurrence of that organism, regardless of its abundance, in the total stomach content. As a result, the difference between the day and night values as presented here is a conservative one.

ate ostracods. These two organisms, together with coenagrionid nymphs, formed the major constituents of the diet.

The results of the 1965 gut content analyses are presented in table 3. In that year *Simocephalus, Cyclops,* and *Alona* were the organisms most frequently eaten during the daylight hours. At night, however, a decrease was noticeable in the consumption of all organisms except *Daphnia* and Tendipedidae. In the case of the *Daphnia,* the quantity eaten at night was more than double that eaten during the day. Under abnormal conditions, such as the addition of well water, the relative abundance of most of the food organisms was drastically altered. Tendipedidae and

Simocephalus retained their normal values whereas the others decreased. During the time that well water was added snails were included in the diet, and the number of non-aquatic insects eaten was increased by a factor of four.

In favor of the hypothesis that the fish moved about in relation to food organisms, rather than temperature, are the clockwise migrations in the pond of *Cyclops* and *Alona*, and the principally northern-shoreline distribution of tendipedids, all of which are important components of the daytime diet of *Gambusia* (table 3). At variance with the hypothesis are the facts that *Cyclops*, *Simocephalus*, and *Alona* in general prefer shallows and *Diaptomus* and *Daphnia* prefer deeper water, and that when these organisms migrate (seemingly in response to sun and shade differences rather than temperature) each organism maintains its preferred bathymetric position. Moreover, when *Simocephalus* does move from one area to another, that movement is counterclockwise. Most significant, however, are comparisons of diurnal and nocturnal feeding activity of *Gambusia* which show a basically adventitious pattern of feeding in which shallow-water organisms are taken in abundance during the day and deeper-water organisms taken in greater numbers at night in relation to movements of the fish to warmer, deeper waters in the pond. Other observations showed that the diet of the population changed several times whereas the pattern of movement of the fishes remained the same through a period of three years.

Such changes in diet were observed between 1964 and 1965 and again during 1965. The latter, involving a change from planktonic forms to non-aquatic insects, demonstrated the lack of correlation between the distribution of food organisms and fish movements. The distribution of insects corresponded with the position of vegetation, whereas the fish continued their normal pattern of movements in relation to changes in surface temperature.

It is clear, nevertheless, that the pattern of movements of the fish, in relation to changes in surface temperatures of the pond is influenced, however slightly, by the absolute abundance of food organisms. It could scarcely be otherwise, considering the similarity in the movements of such food organisms as *Cyclops* and *Alona* which, between 10 A.M. and 4 P.M., follow more or less the same migratory routes as the fish. The responses of the fish to temperature and food are in a changing balance. Probably, however, the adventitious nature of the feeding activity of the fish must permit the thermotactic responses to assume overriding importance at various points during the daily migratory schedule of the fish.

Behavior of Females and Young in Reproductive Season

General Pond Behavior: In the way that feeding patterns exert an influence on the diurnal movements of the fish in response to changing surface temperatures, so do mating and reproductive activities of the individual fish affect the behavior of the population as a whole. It was indicated above how gravid and non-gravid females select different microniches with respect to substrate color and water depth, after reaching the northern, sandy shoreline of the pond. Mention also was made of a periodicity in microniche selection related to changes in the reproductive state of the females and the presence or absence of large numbers of courting males. Widespread courtship and mating activities occur only once a year at the beginning of the reproductive season, whereas changes in microniche selection by the females may be expected to occur as often as new broods are produced from a single original insemination.

The evidence so far compiled for the particular population we have studied indicates that widespread courtship and mating activity begin early in May and are reduced to a much lower (or at least less evident) level by early June. One would expect that males and females born during June and maturing in early August would contribute a second peak of mating activity to the population in late summer, but such a marked increase in sexual behavior has not been noted. A possible explanation for the absence of a marked increase in sexual activity may relate to the broad period over which individual 0-year class males and females become ready for reproduction. In contrast, overwintering fish are all previously differentiated sexually and are more or less synchronized in their first seasonal mating activities by climatic and other factors.

In the pond environment presently being studied the reproductive season in *Gambusia* extends over a period of approximately six months, from early May until sometime in October. Adult females collected in early October of 1964, for example, were found to contain advanced embryos, and a few newborn young had been seen occasionally in the pond in late October over the four-year period of the study. In addition, adult females collected in early November were found to contain small ovaries with oocytes in early stages, but no mature eggs. The earliest recorded appearance of young was June 3, 1965 when hundreds of newborn fish were seen along the northern shore of the pond. In other years the first wave of young appeared on June 10, 1964 and June 13, 1963. Allowing a three-to-four-week period for gestation would require mature eggs to have been developed by the first or second week in May.

The estimate of development time is based on records of the actual successive waves of young seen in the pond in 1964 and 1965, particularly those waves that appeared during the first half of the summer. During 1963 the average time between the more or less synchronously produced broods of overwintered females was 31.5 days (broods on June 13, July 14, August 17), during 1964 the average elapsed time between the first three broods was 28 days (broods on June 10, June 27, August 5), and during 1965 the average elapsed time was 24 days (broods on June 3, June 29, July 21). In all years, after midsummer, the young from the first broods of the season were mature and contributing their own young to the population. The young appearing any time after midsummer were derived from adults of different year classes and these were not, of course, synchronized. Hence, new young fish were seen to appear in the pond continuously from late July or early August until sometime in October.

To provide some idea of the rate of growth and maturation of fish born in early June in the pond, 20 newborn fish were collected and measured and each week thereafter 20 additional fish were removed and similarly measured. The general procedure was followed until the fish were fully differentiated sexually. The procedure was also carried out for the second wave of young which, on a size basis, could always be clearly separated from other fish in the pond. In 1964 the average rate of growth (see also table 4) for the first 20 days of life was 0.35 mm. per day for females. In 1965 the average growth rate for the first 20 days was 0.69 mm. per day. The 1964 fish attained in this time a size of 15.0 mm. and those measured in 1965 attained a size of 21.8 mm. The differences between the 1964 and 1965 young in growth rates and

TABLE 4

COMPARISON OF GROWTH RATES (IN MILLIMETERS)[a] FOR THE FIRST WAVE OF YOUNG *Gambusia* IN EARLY JUNE OF 1964 AND 1965

	1964				1965			
	20 days	Mean Length	44 days	Mean Length	20 Days	Mean Length	44 Days	Mean Length
Female	0.35 day	15	0.34 day	23	0.69 day	21.8	0.73 day	40
Male	—	—	—	—	0.47 day	17.4	0.34 day	23

[a] Rates are calculated per day for the first 20 and the first 44 days of life and are based on the average length of 170 fish measured over comparable 44-day periods in the two years. The average length of fish at the end of each growth period is indicated.

sizes attained after 20 days of life presumably were related to differences in the size of the overwintered populations (see Materials and Methods for description of the 1965 restocking). In spite of differences in growth rates and final sizes attained, during both years sexual differentiation had begun when the fish reached 14 mm. All of the fish so studied had completed sexual differentiation by the twenty-first day of life.

The data above show clearly the profound effects on individual growth rates of initial population density. Under ideal conditions *Gambusia* is capable of colonizing a small pond or lake with great rapidity. A number of factors operate to limit population size, however. Presumably the availability of food is one of them and may well account for the differences in growth rates and final sizes attained by 1964 young-of-the-year, which had to compete with a large overwintered population, as compared with 1965 young which shared the pond with only 26 adults. Some idea of the reproductive capacity of the species may be gained by estimating the number of young that would be added to the pond during a single season beginning with 10 adult females, and assuming no mortality, and ample food and space. With an average brood size of 50 young, an average maturation rate of young-of-the-year of 20 days, and an average brood interval of 25 days for all mature fish over the six-month breeding season, the population, in October, would be expected to total 4,802,500 individuals. Inasmuch as the water volume of the pond at its maximum depth of 3½ feet is relatively small (the surface area not exceeding 5000 to 6000 square feet), mortality of young and adults in the pond must be very great and various biological factors probably inhibit reproduction itself. The highest crude field estimates of population size based on five-times-daily counts of fish throughout the pond over a three-year period never exceeded 10,000 individuals. If no biological factors were acting to inhibit reproduction or maturation, the calculated mortality of the hypothetically possible population would be 99.8 per cent.

LABORATORY ANALYSIS OF BEHAVIOR: An outstanding feature of the behavior of *Gambusia* under pond conditions is the preference of gravid females during the breeding season for the light, sandy bottom of the northern shoreline of the pond. Substrate preferences of non-gravid females are usually non-specific or are for the darker muddy patches at the edge of or beyond the sandy slope. Moreover, newborn young appear to be almost entirely restricted in distribution to the sandy bottom of the northern shoreline, even during the night when the adult population moves to the deeper waters in the south of the pond.

Experiments to test the responses of adult females and to a lesser extent of young *Gambusia* to dark and light substrates and to differences in water depth in relation to substrate color were conducted.

In 1964 three 8-gallon aquariums were used to test the preference of individual fish for substrate color. Black and white sands were used as contrasting colors. The black sand was prepared by coating the white sand with India ink and incinerating it to render it permanently black.

A constant depth was maintained in each tank. Number 1 was filled to a depth of 10 inches; number 2 to a depth of 5 inches, and number 3 to only 1 inch. A 100-watt incandescent bulb was suspended 1½ inches above the rim of each tank. Fish were introduced over the black and white interface of each substrate and observed for a 10-minute period. Preference was determined on the basis of percentage of time spent over each color. A total of 369 adult females were tested in this manner. Each was sacrificed immediately following the experiment and the gonads preserved in order to determine the stage of embryonic development of the young.

In 1965 a modification of this experiment was conducted to determine the preference of individuals for contrasting substrate colors in conjunction with a depth gradient. Eight 23-gallon tanks were used. Each measured 36 by 12 by 12 inches and was prepared as in figure 17. Variable depth was effected by tilting a tank on a wooden frame inclined at an angle of 4 degrees. The depth in each of the tanks numbered 1, 2, 5, 6, and 7 ranged from ½ to 3 inches. Tanks numbered 3 and 4 had a depth gradient of 3¼ to 6 inches. Sand was prepared as in the 1964 experiments. The two colors were separated by a strip of glass ½ inch high, and a plate of glass was placed on the surface of the sand. This prevented the mixing of the colors and minimized bottom disturbances when introducing and removing fish.

Two full-length reflectors were suspended over each tank 16 inches above the surface of the water. Each reflector was fitted with a 30-watt Westinghouse Warm White fluorescent bulb.

Fish were tested one at a time by introducing them over the black and white interface of the bicolored substrate. The position of each fish was recorded over a 10-minute period at one-minute intervals. During the experiment, all room lights were turned off and observations made from a distance to avoid disturbing the fish.

Each age group was tested in all eight tanks each week. The oldest young-of-the-year (born June 3) were tested from the age of one week to the age of 12 weeks. The second-wave young (born June 29) were tested from the age of one week to the age of seven weeks. Third-wave young were tested only at the age of one week. Each fish was used for

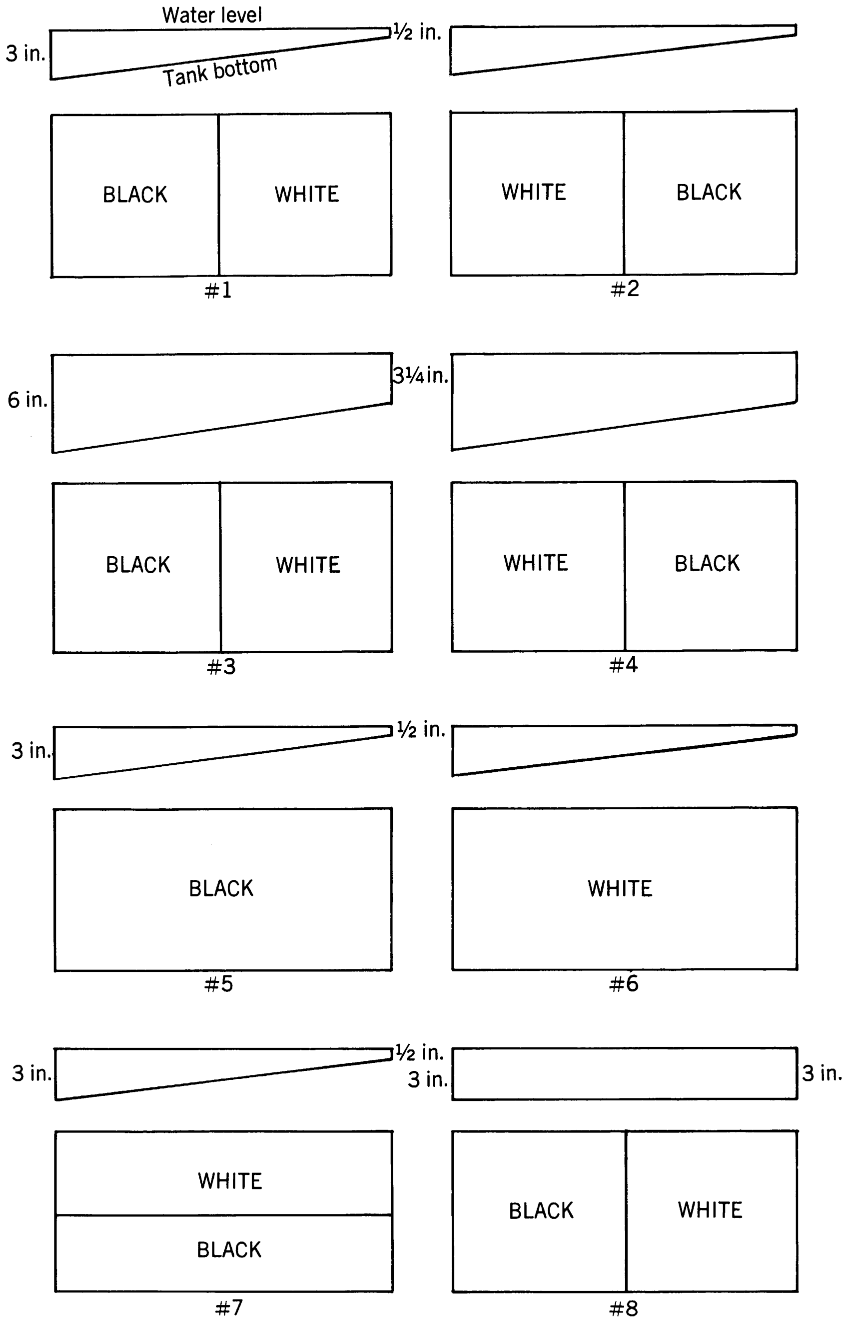

FIG. 17. Arrangement of substrate color and water depth in eight experimental aquariums used in the 1965 phase of substrate-preference tests.

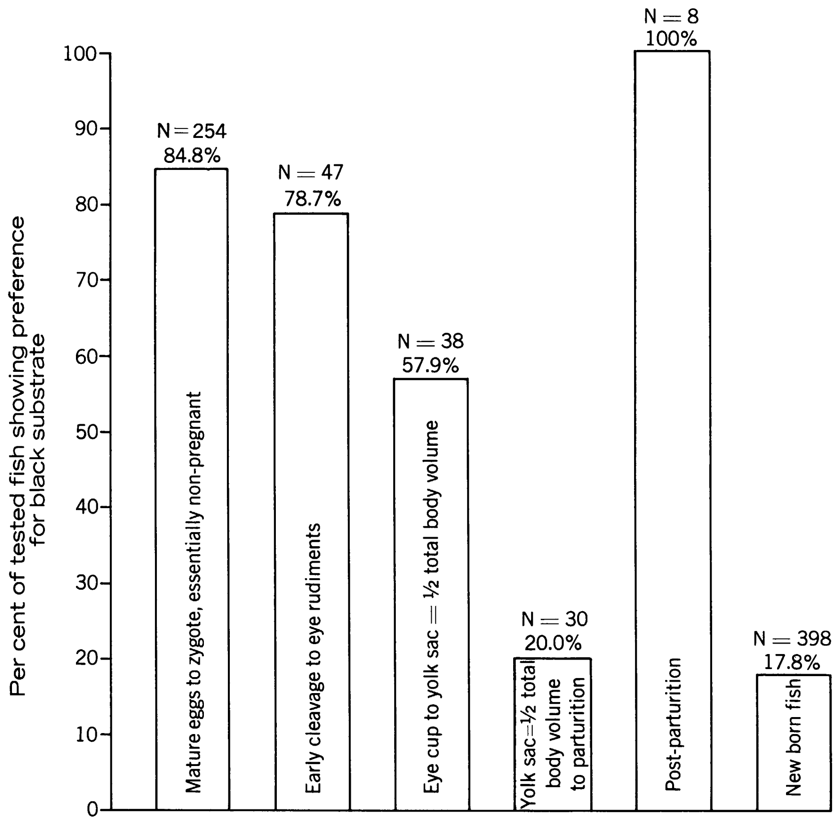

FIG. 18. Laboratory responses to substrate color in young *Gambusia* and in adult females at various phases of the reproductive cycle during 1964.

only one experiment in one tank before being returned to the pond. Only young fish and adult females were tested.

Once a week two females from a test sample were sacrificed from each age group and the gonads were removed and preserved. The stage of development of the embryos in these samples, as well as the external appearance of females in each age group was noted. In this manner the adult females in each age group could be assigned an estimated position in the reproductive cycle.

As stated above, 369 females were tested during the 1964 phase of this experiment. Each fish was sacrificed immediately following the test and placed in a group according to four arbitrarily determined stages of embryonic development. The first stage was represented by females with embryos from late cleavage to the development of an embryonic axis with eye rudiments. The second group included stages from the first appearance of the eye cup to the point at which the yolk sac was half of the total body volume. The third stage was represented by females from stage two to the point of parturition. The fourth group was made up of non-pregnant females. These contained either mature eggs or zygotes in which there were as yet no signs of cleavage.

The response to substrate color recorded for these groups showed a rapid decrease in preference for a black substrate as embryonic development advanced. Figure 18 shows this change as recorded in the 1964 experiments. It also shows the non-pregnant females and a post-parturition group. The latter included seven females that dropped their young in the laboratory and were tested immediately thereafter.

Table 5 shows the cyclic variations in substrate color choice as correlated with fluctuations in the reproductive cycle. The table represents the total responses in all tanks in the 1965 experiments which had bicolored substrates. When the tanks were examined individually, the results showed distinct differences in the choices made in each. Fish tested in tanks numbered 1 and 2 showed a high black and white preference respectively, corresponding to the deep side of the tank. Fish in tank number 3 showed a high preference for the black substrate (92% black), whereas those in number 4 showed no preference (49.5% black). Results obtained in tanks numbered 7 and 8 showed three successive peaks in black preference and two in white preference after the fish had attained sexual maturity (table 5; fig. 19). Immature fish were found to have responses similar to those of pregnant females. In figure 19 the relatively small preference differential between pregnant and non-pregnant females is because the data are based on age groups in which the individuals are not all in exactly the same phase of the reproductive cycle.

In the 1965 study (table 5) no correlation between depth preference and stage of the reproductive cycle of the fish is evident. Analysis of depth preference of fish in each tank indicates a slight preference for deep water in tanks number 1, 2, 5, 6, and 7. Only in tank number 3 did the fish show a definite and positive response to deep water. In the equally deep tank number 4, absence of choice by the fish may indicate that fish entering the deep side were often repelled by white substrate. Similarly, a preference for the deepest water in tank number 3 may have been reinforced by the dark substrate of that side.

In general, the results of these tests demonstrate that about 80 per cent of the young and 80 per cent of the adult *Gambusia* females in ad-

TABLE 5

SUBSTRATE PREFERENCE OF FEMALE AND YOUNG *Gambusia*, 1965 LABORATORY EXPERIMENTS [a]

Tanks No. 1–4

Age in Weeks	Number of Fish Occurring in Each of 10 Frequency Classes (Number of Positive Responses out of 10): White Substrate 10	9	8	7	6	5	4	3	2	1	Black Substrate 1	2	3	4	5	6	7	8	9	10	No. Fish Tested	Percentage of Occurrence over White Substrate	Percentage of Occurrence over Black Substrate
1	3[b]	1[c]	1	1	1	—	—	1	—	1	1[c]	1	1	1	—	—	1	—	1	1	12	53.3	46.7
2	2	1	9	6	4	10	7	6	13	11	1	9	6	4	10	7	6	13	11	10	79	37.9	62.1
3	9	2	3	4	4	3	6	6	5	14	2	3	4	4	3	6	6	5	14	16	72	36.8	63.2
4	2	3	4	—	3	7	5	7	11	9	3	4	—	3	7	5	7	11	9	9	60	34.0	66.0
5	5	2	3	3	5	4	5	8	11	13	2	3	3	5	4	5	8	11	13	17	76	31.8	68.2
6	8	3	3	2	4	3	5	3	16	15	3	3	2	4	3	5	3	16	15	18	80	32.5	67.5
7	4	2	4	8	8	2	3	5	9	13	2	4	8	8	2	3	5	9	13	12	70	37.4	62.6
8	1	2	2	3	6	3	7	3	7	3	2	2	3	6	3	7	3	7	3	3	40	42.5	57.5
9	4	2	1	—	4	6	3	—	8	6	2	1	—	4	6	3	—	8	6	19	53	29.0	71.0
10	3	1	8	3	1	5	6	5	8	5	1	8	3	1	5	6	5	8	5	11	56	38.4	61.6
11	4	3	3	2	6	3	2	3	2	3	3	3	2	6	3	2	3	2	3	9	40	45.0	55.0
12	3	—	2	—	—	4	2	1	4	11	—	2	—	—	4	2	1	4	11	9	36	26.7	73.3

TABLE 5—(*Continued*)

Tanks No. 7–8

Age in Weeks	Number of Fish Occurring in Each of 10 Frequency Classes (Number of Positive Responses out of 10): White Substrate 10	9	8	7	6	5	4	3	2	1	Black Substrate 1	2	3	4	5	6	7	8	9	10	No. Fish Tested	Percentage of Occurrence over White Substrate	Percentage of Occurrence over Black Substrate
1	2	—	2	1	1	1	—	—	1	—	—	2	1	1	1	—	—	1	—	—	8	70.0	30.0
2	10	3	3	1	—	2	2	2	1	1	3	3	1	—	2	2	2	1	1	1	26	71.2	28.8
3	1	2	3	2	6	3	4	5	1	7	2	3	2	6	3	4	5	1	7	6	40	39.3	60.7
4	2	—	2	2	4	5	2	2	3	3	—	2	2	4	5	2	2	3	3	1	26	46.9	53.1
5	1	1	1	2	3	2	4	7	4	7	1	1	2	3	2	4	7	4	7	7	39	31.0	69.0
6	2	2	2	5	4	2	4	3	5	4	2	2	5	4	2	4	3	5	4	7	40	40.5	59.5
7	4	3	4	1	4	5	1	5	3	1	3	4	1	4	5	1	5	3	1	1	32	56.6	43.4
8	2	2	1	4	5	2	4	4	3	6	2	1	4	5	2	4	4	3	6	7	40	38.5	61.5
9	1	—	1	2	2	2	1	6	4	6	—	1	2	2	2	1	6	4	6	7	32	28.1	71.9
10	4	3	3	2	2	2	1	2	2	2	3	3	2	2	2	1	2	2	2	—	23	62.2	37.8
11	1	5	2	1	2	2	1	1	1	—	5	2	1	2	2	1	1	1	—	—	16	68.1	31.9
12	—	—	1	1	2	4	1	1	1	3	—	1	1	2	4	1	1	1	3	6	20	29.5	70.5

[a] The position of each individual was recorded once each minute over a 10-minute observation period.

[b] The number 3 represents the three fish, age one week, that were recorded over white substrate at each observation during the observation period.

[c] The number 1 represents the single fish, age one week, that was recorded over white substrate on 9 of the 10 observations during the observation period. The same fish is recorded as occurring once over black substrate (see second entry for this footnote).

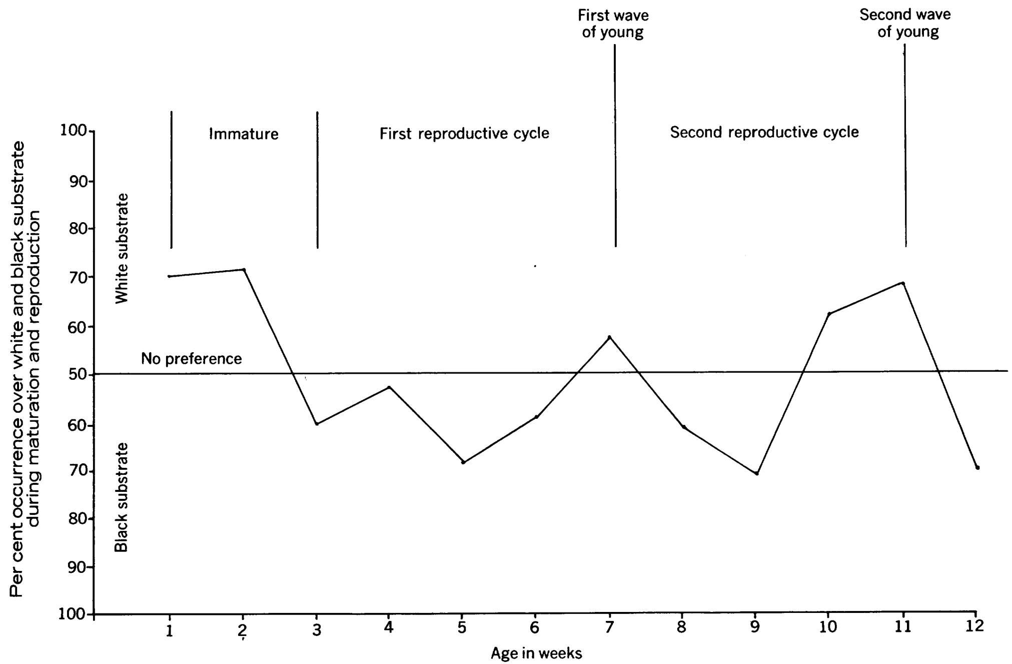

FIG. 19. Percentage of tested *Gambusia* in each of 12 age groups during 1965 that showed a 60% or greater preference for black or white substrates at constant depth (tanks numbered 7 and 8). The reproductive state of the 325 fish on which this figure was based was estimated. See pages 31–35 and table 5, and compare with the 1964 observations, summarized in figure 18, in which the actual reproductive state was known.

vanced pregnancy will spend more than 60 per cent of the observation period over a light substrate under conditions of constant water depth (see fig. 18). Conversely, about 80 per cent of the adult females in early pregnancy or with unfertilized eggs, under the same experimental conditions, will spend more than 60 per cent of the observation period over a dark substrate. Females in mid-pregnancy, under these same conditions, spend approximately equal amounts of time over light and dark substrates. These responses to substrate color, in the laboratory, are cancelled out by offering the fish a choice of water depth in conjunction with a choice of substrate color, under which conditions the female fish usually spend the bulk of the observation period in the deepest possible water irrespective of substrate color.

Behavior of the fish in the laboratory is consistent with the observed pond behavior. As noted above, females in advanced pregnancy and young fish as well, spend a major part of the daylight hours over the light-sand bottom of the northern shore of the pond, whereas non-pregnant females exhibit no preference for the light bottom. In the pond, adult females and young that are concentrated over a light-sand bottom, when disturbed by an observer on the shore, invariably seek deeper water (which coincides with a dark substrate) but soon return to the original position on light sand when the disturbance disappears. The fright reaction of pregnant females would seem to imply only that the fright response overrides a physiologically induced response to a light substrate. In the laboratory, when conditions are seemingly uniformly stressful, the choice of deeper water is expected whenever that choice is available. Nevertheless, the importance of the physiologically imposed substrate color selection can be demonstrated when the opportunity for seeking refuge in deeper water is eliminated. In fact, we suppose that if the stressful aspects of laboratory procedure could be further reduced or eliminated, the responses of pregnant females to light sand and of non-pregnant ones to dark bottom would be more nearly an absolute response rather than only a 60 per cent or greater average response to the appropriate background shade.

CONCLUSIONS

The results of this study of the pond ecology of a northern-hardy strain of the viviparous mosquitofish, *Gambusia affinis,* have shown that during the non-winter months, diurnal migrations of fish around the pond and even the fine adjustments of their position in any sector of the pond are strongly influenced by surface temperatures. Throughout the months of greatest activity, the fish select the highest temperatures available below a maximum of 33° C. The individuals appear to feed adventitiously, and the distribution of food organisms is not known to alter appreciably their daily clockwise movements around the pond. During the reproductive season, from May to October, the midday distribution of adult females in the pond, when their diurnal migrations bring them to the northern, shallow, sandy shoreline of the pond, is slightly but definitely influenced by reproductive condition. Females in advanced pregnancy spend most of their midday sojourn on the northern shoreline directly over the light-sand bottom. Females that have just given birth or are in early pregnancy assume a position on the northern shoreline in somewhat deeper water beyond the edge of the sand slope over dark mud bottom. Laboratory study confirmed the differences in substrate preference by females in different states of reproductive readiness, and further showed that females in mid-pregnancy spend more or less equal amounts of time over light and dark substrates.

Water temperature appears, therefore, to be the overriding stimulus that regulates the gross diurnal movements of the population. If it is true in *Gambusia,* as it is for so many other poikilotherms, that sexual maturation and reproduction rates are greatest at the highest temperatures, it may be inferred that colonization of a habitat by this species might be enhanced by its characteristic of maintaining a position at the higher temperatures. Superimposed on the dominant diurnal migratory periodicity, in response to shifting surface temperature gradients, is the more or less monthly periodicity of changing microhabitat selection in response to changes in reproductive physiology. The selection by near-term females of a light sand substrate may correspond to a general tendency to move inshore into shallow water for parturition. The extremely shallow shoreline habitat may, within the natural range of this species, form a kind of nursery that in general affords a proper feeding ground and protection for the newborn against larger aquatic predators that customarily avoid or cannot enter the shallows. Since adult fish can color-adapt to any shade of dark or light substrate, only the rate of accommodation would be expected to evoke an avoidance or preference for a particular type of bottom. Previously color-adapted *Gambusia,* as suggested by the work of Sumner, will not readily cross a contrasting background. Avoidance of contrasting backgrounds that would promote predation may, therefore, be expected occasionally to influence the daily cyclic movements within the over-all habitat. Finally, the distribution of food organisms may be expected to exert only the slightest influence on diurnal movements, as indicated by the adventitious feeding pattern of this species. Freedom from the requirements of a specialized diet, a well-defined habitat selection for parturition that favors survival of newborn fish, and a positive thermotaxy that might enhance a great inherent reproductive capability, may, upon further study, prove to be factors of principal importance to the local and geographic success of this fish species in nature.

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SUMNER, F. B., AND P. DOUDEROFF

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