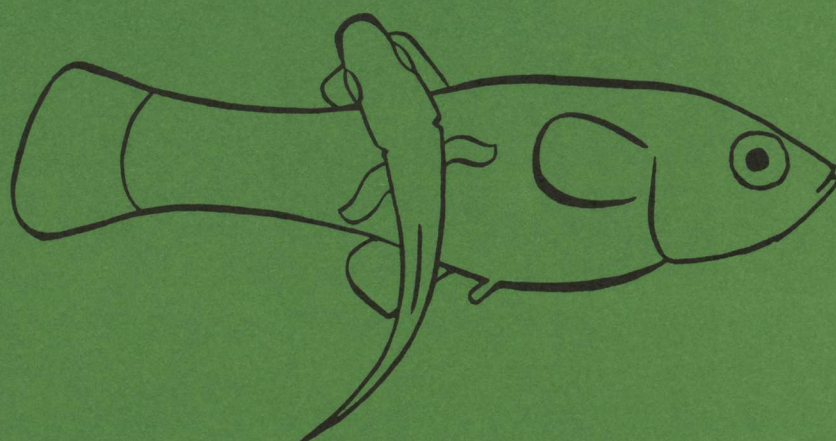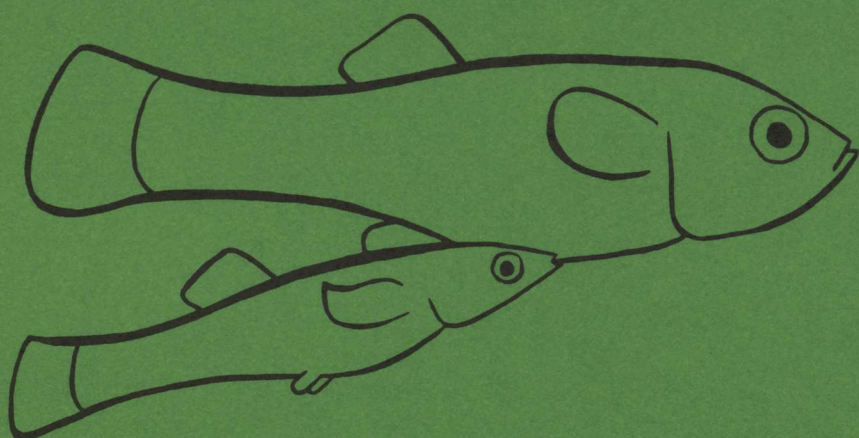3.00

BULLETIN 19

TEXAS MEMORIAL MUSEUM

Competition and Isolation Mechanisms in the
Gambusia Affinis X *G. Heterochir* Hybrid Swarm

by Clark Hubbs

THE UNIVERSITY OF TEXAS AT AUSTIN

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Frontispiece: Clear Creek headwaters

ILLUSTRATIONS

LIST OF TABLES

Clear Creek Headwaters

Upper left: upper pond from cliff above station A5. Upper right: upper pond from cliff above station B1. The traps in B1, B2, and B3 can be seen in the fore-ground. The break in the dam is near the far shore and the spillway near the two bushes on the dam. Middle left: the headwaters of Clear Creek. The spring adjacent to A1 is on Dr. Crespo's right and the one between A4 and A5 flows from the right

COMPETITION AND ISOLATION MECHANISMS IN THE
GAMBUSIA AFFINIS × *G. HETEROCHIR* HYBRID SWARM

By Clark Hubbs

Introduction

The effect of the physical environment on a species has been studied extensively, but an analysis of the interactions of two closely related species in their natural environment may provide considerable insight into community structure. Most sympatry is between distantly related species so that the probability of niche overlap in all parameters is small. Because near relatives have diverged recently the opportunity for chance differentiation of adaptive peaks is limited.

This study of the interactions of two fish, *Gambusia affinis* and *G. heterochir,* is further complicated by the presence of numerous hybrids in all areas of sympatry. Although many putative fish hybrids have been recognized in nature (Hubbs, 1955), most are isolated examples that could have resulted from chance meeting of gametes in nature. Accidental occurrence cannot account for the *Gambusia* hybrids reported here because of their high frequency and, more significantly, because internal fertilization of eggs is required. It is therefore apparent that the hybrids must have resulted from a breakdown of behavioral isolation mechanisms, the causes of which are postulated in this study.

The hybrid swarm was first sampled 22 February 1953 (Hubbs, 1957a); and all studies show more than 10% of the adults in the swarm area are hybrids. With such a high frequency lasting for at least 17 years, fusion of the parental species would be expected unless the respective gene pools were distinctly and separately integrated. Some data are available to show how each unit maintains its genetic integrity.

The Study Area

The hybrid swarm lives in the headwaters of Clear Creek, Menard County, Texas, 16.7 kilometers west of Menard; the creek begins with a series of springs at the base of a limestone cliff (frontispiece). An earth-concrete dam constructed in the 1880's impounded most of the spring sources and diverted some of the water for household and irrigation purposes. When I first visited the area in 1953, irrigation had ceased and the dam was in poor repair. Several poplars that had been planted in the earth fill between the concrete sides had fallen, damaging the dam. The most serious break was on the east end where a small trickle permitted fish exchange (fig. 1). Similarly, fish could pass through the spillway in both directions. During all sampling times, most of the hybrid swarm was concentrated above the break on the east end of the dam (Hubbs, 1959), and few *G. heterochir* have been obtained downstream from the dam.

About 40 years after the upper dam was constructed (and 30 years before the first sampling) two more dams were constructed, one and two kilometers east of the upper dam. Each impoundment reached to the base of the next dam upstream, and the upper dam impounded Clear Creek up to the base of the original earth-concrete dam (fig. 2). This impoundment covered the irrigated area so that the diversions were limited to those for domestic use. The area of the second impoundment has had dense *Gambusia* (especially *affinis*) populations on all visits.

At the first visit the banks of the lower pond were lined with large pecan trees and the shallow areas filled with cattails. During the 1957-58 winter, introduced *Nutria* decimated the cattails and prior to 1967 virtually eliminated the pecans. These changes in the environment seem to have had minimal effect on the relative fish densities.

Because the downstream dams are out of the study area, subsequent reference to a dam signifies the earth-concrete dam constructed in the 1880's.

Methods

The fish were collected in plastic minnow traps similar to those reported by Deacon *et al.* (1964), Deacon and Wilson (1967), and Hubbs *et al.* (1967). The traps are 42 cm long, 22 cm in diameter in the middle, and 16 cm in diameter at both funnel ends. The funnels narrow to 28 mm but have small perforations through which small-to-medium-sized *Gambusia* may pass. The sides of the traps have the same type of openings covered with fine plastic netting (less than 1 mm openings). The traps were baited with 1 tablespoon of Ken-l Ration dog food.

The study area was visited at approximately monthly intervals: 6 September, 11 October, 5 November, 3 December 1967; 7 January, 4 February, 3 March, 31 March (hereafter called April), 5 May, 1 June, 7 July, 1 September, 5 October 1968. The traps were set between 1000 and 1200 hours and removed between 1700 and 1900 hours (Central Standard or Daylight Savings Time depending upon the season). During the September 1967 visit, the traps were not baited until about 1400 hours at which time those fish already trapped were removed and preserved in 10% formalin. On the other 13 trips, the traps were not disturbed between setting and

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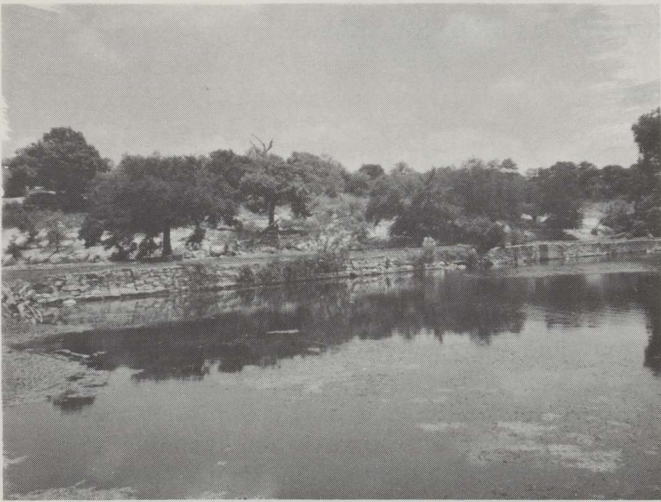
Fig. 1. Earth-concrete dam and other stenothermal areas. Upper left: upper dam from station F. Upper right: the west end of the dam with stations C1 and C2 in the foreground. Middle left: station G in foreground; station E1 is beside fallen tree halfway to the boathouse in background. Middle right: the break in the dam. The current can be seen in the center and the lower stone wall has large holes permitting *Gambusia* to pass through. Lower left: E series of stations from E1 on west shore. Lower right: station Q.

Fig. 2. Eurythermal areas at Clear Creek. Upper left: station E11 in foreground; station G is adjacent to the stone house in the background. Upper right: station K in foreground. Middle left: station L in foreground. Middle right: station P in foreground. Lower left: station H in foreground. Miss Boecker is beside downstream diurnal. Lower right: station 0 is in foreground.

3
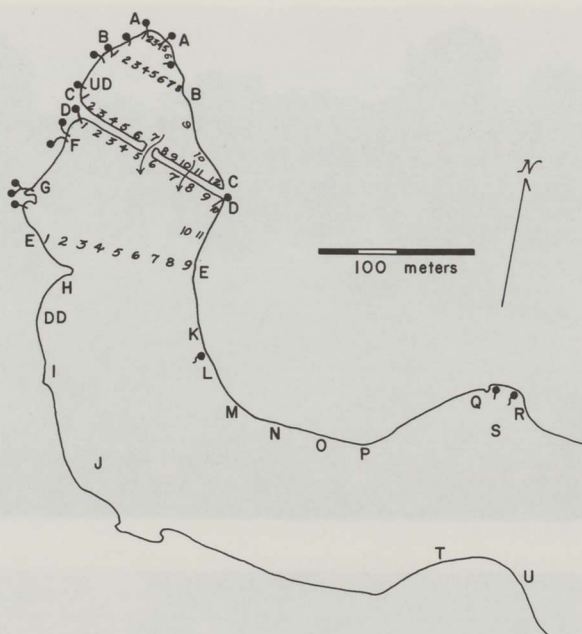
Fig. 3. Locations of traps at Clear Creek. The springs are designated by dots with adjacent outflows. See text for descriptions of trap locations. DD = downstream diurnal. UD = upstream diurnal.

removal. All vertebrates and large invertebrates in the traps were preserved and returned to Austin for examination.

A variety of approaches was carried out to contrast species and locality. Specific methods for each are described in appropriate sections of this report.

Krumholz (1948 and 1963) made comparable studies on populations of *Gambusia* from two regions but recorded size as total length. Standard length is used in this investigation as in most studies of fish biology. We obtained a sample of the descendants of the fish Krumholz studied in 1948 and found that the conversion ratios were 0.78 for males and 0.79 for females, or, to simplify, standard length is approximately 4/5 of total length.

Fish were measured to the nearest tenth of a millimeter and were pooled by millimeter; therefore, fish listed as 20 mm were 20.0-20.9 mm standard length.

The Stations

The traps were set in approximately the same locations during the 14 monthly sampling times. Less precision was possible at a distance from shore, but the variations are not likely to seriously distort the results. Water level changes occasionally necessitated a horizontal movement of inshore traps, especially if the "standard location" was on the bank during low water.

Five series of traps extended across the stream; 5 were on the southwest shore and 8 on the northeast shore (fig. 3). Traps were also occasionally placed at 8 other localities.

The upstream series of single traps, A1-A5, was placed adjacent to and between the two springs that begin the stream. A4 and 5 are on the sides of a spring run, A1 and 2 in dense *Ceratophyllum* and A3 in a 1 m X 1 m opening which seems to be maintained by *Nutria*. A6 was sampled in August 1968 only. Six traps were used.

The B series of traps was located midway in the upper pool. B1 and 2 were single traps in shallow water and B3-B8 in water 1-2 m deep. Each of those 6 stations had a surface and a bottom trap. All were placed in thick *Ceratophyllum*, and/or *Ludwegia*. The B series of traps was also sampled the night following the daytime collections. B9 and 10 were set in August 1968 and each contained 6 traps set in shallow water.

The C series of single traps was located along the upstream edge of the dam. Stations C1, 2, 10, 11, and 12 were in shallow water and the others floating among the *Ceratophyllum*. Those in C10, 11, and 12 were in the shade of a large live oak tree and the *Ceratophyllum* was often sparse. Stations 10 and 11 were on opposite sides of the break in the dam. Each station had one surface trap.

The D series of single traps was set along the downstream edge of the dam. Stations D1, 4, and 10 were in shallow water while others were set to float on the surface. All but station 3 were placed in *Potamogeton* beds which often were dense at station 2. Stations 8 and 9 were on opposite sides of the break in the dam. Stations 1 and 10 were in small spring runs.

The E series of traps was set across the stream at a comparable distance from the dam as the B series. Stations E1, 9, and 11 were in shallow water, 1 in an area with much shade and minimal aquatic vegetation, 9 in a *Nutria* channel with little vegetation, and 11 in dense *Potamogeton* except when *Lepomis* were spawning. Stations 2-8 and 10 had surface traps floating in the *Ceratophyllum* and a trap on the bottom. The E series of traps was also sampled the night following the daytime sampling. The floating vegetation at B and E is reasonably dense – only 1 floating trap floated from its original site despite occasional strong winds.

Each of the 13 stations along-shore had 3 traps.

Station F was in the outflow of 2 of the downstream springs. Watercress was dense and many limestone boulders were scattered along the muddy bottom.

Station G was in the outflow of 2 downstream springs and adjacent to a stone spring house. Watercress was dense. Station G was also sampled each following night.

Station H was adjacent to a floating boat house and *Ceratophyllum* and/or *Potomogeton* was abundant. Station H was sampled each following night.

Station I was on a very gradually sloping mud flat. Typically the traps were set adjacent to a series of lily pads.

Station J was offshore on a shallow sloping area near an old fence post among lily pads.

Station K was near some dead pecan trees. The traps were set in a shallow depression between the trees and a series of lily pads. Three small springs were adjacent to the lily pads but not within the area trapped. Station K was also sampled each subsequent night.

Station L was on a gradually sloping shore. The traps were in 10-20 cm water 10 m offshore in *Potomogeton*. When possible they were placed in *Nutria* channels. Station L was also sampled each subsequent night.

Station M was similar to station L except that traps were not set at night.

Stations N and O were set near some lily pads. The areas also had dense *Potomogeton*.

Station P was set on a point with a 15 cm offshore shelf. The traps were set along the shelf among sedges.

Station Q was near the eastern spring among sparse growths of *Ceratophyllum* and was in the shade of a willow tree.

Station R was among lily pads and *Ceratophyllum* near the eastern spring. The station was adjacent to a rock wall with a 15 cm shelf at the water edge.

Station S was offshore between Q and R and in the middle of the flow of the eastern spring. It had 6 traps set only in August 1968.

Stations T and U were along the southern bank in lily pads. Each had 6 traps set in August 1968.

The stations above the earth-concrete dam had firm bottom of limestone origin. Those along-shore below the dam were also reasonably firm except for I, J, K, L, M, N, O, and P, in which the mud was deep and flocculent. In such habitats aquatic vegetation was the only foothold available to prevent investigators from sinking one meter into the mud below one decimeter deep water.

Thermal Stability

The upper pool is inhabited extensively by *Gambusia heterochir* and the hybrids; it differs from areas typical for *G. affinis* in many environmental parameters. Biologically, the upper pool has a different morph of *Ceratophyllum* and amphipod than those dominant in the lower pond. Chemically, waters in and adjacent to the spring outflows have lower pH than do those at a distance. Physically, spring waters are more consistent thermally than are those exposed to ambient temperature. The last was selected for analysis because it is easy to quantify and undoubtedly reflects the other environmental gradients. For example, stations with comparable temperature readings have comparable flora.

Readings were obtained from each regular along-shore (easily reached) trapping station on the visits
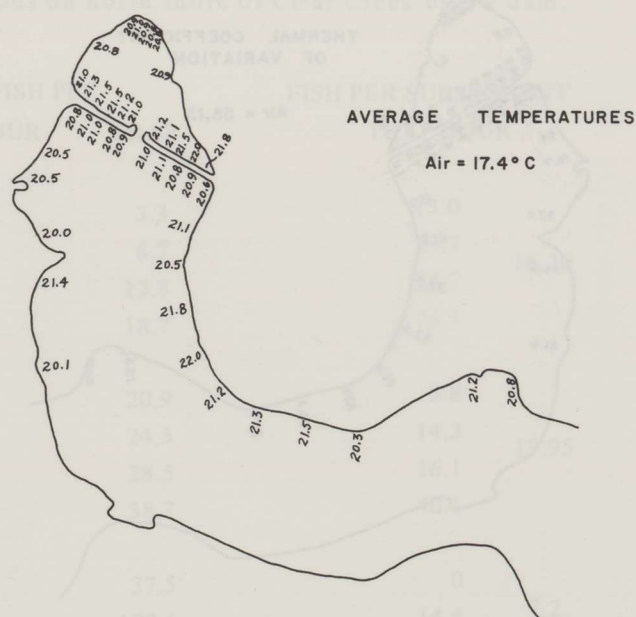
Fig. 4. Average temperatures for sampling stations at Clear Creek.

November 1967 through October 1968. Two readings were obtained at about 1400 and 2200 hours. On warm sunny days, water temperatures varied with depth; on one occasion the temperature was 5° C colder at 5 cm depth. Because *Gambusia* typically inhabit surface waters, the temperatures reported are from the surface centimeter. Differences in average temperature (fig. 4) were minimal and did not follow patterns of *Gambusia* distribution. All stations were about 3 to 4° C warmer than ambient, in large part because they were often obtained in sunlit water. The cooler stations, E1 and I, were on the west bank and had afternoon shade; the warmer ones, D11 and L, were on the east bank and exposed to afternoon sun.

The association of temperature with biota is real but it is the thermal variation that is critical (fig. 5). The stations in the upper pool had thermal coefficients of variation ranging between 1.83 and 18.80, whereas those in the lower pool away from spring outflows were above 30. Biological and chemical conditions at those thermally stable stations in the lower pool were similar to those in the upper pool. For example, stations Q and R resembled C1 and C12 thermally, chemically, and in flora; the area between each pair was also similar.

The stations in the lower pool are divided artificially into two groups based on their thermal variation, with one group being equivalent to those in the upper pool and the other having great thermal variation. For example, D-G and Q + R (and S) are considered thermally stable, whereas H-P (and T and U) are considered thermally variable.

Capture Rates

If the traps were of infinite size the capture rate
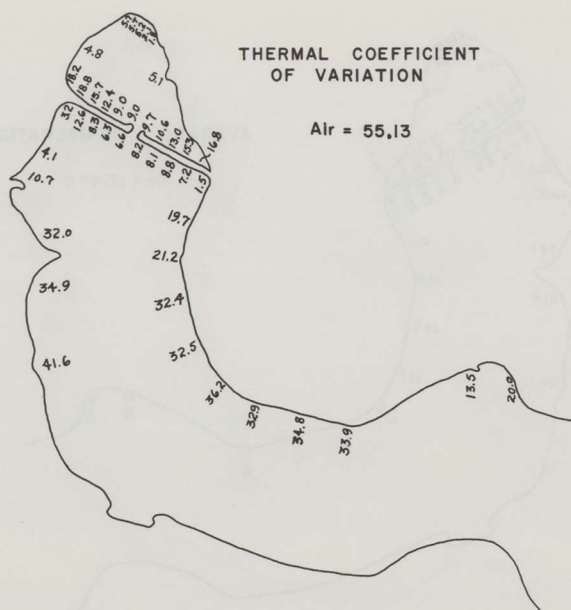
Fig. 5. Coefficients of variation of temperature at Clear Creek.

should be linear with time, but with a reasonably small internal volume an increase in the number of fish in the trap increases the probability that fish will escape. Several tests were designed to determine the relationship of numbers of fish captured to length of trapping time. A series of tests, containing 8,460 fish taken between stations K and N, may provide the best data. Thirty trap locations were set up in 3 replications of 10 designs. Each site was sampled for 9 hours. The basic design was to have 8 stations each with a trap interval of 15, 30, 45, 60, 75, 90, 105, and 120 minutes. Each time interval was also set up so that each would be removed first at one station, second at another, etc., and so that when possible two time intervals at one station did not occur in the same sequence as they did at another station. Several patterns were made up and then tested to determine if by chance one trap time interval emphasized one clock time, and only those that had minimal overlap were used. One of the 10 stations had a trap set for the entire 9 hour sampling time, and 2 more shared one of the shorter sampling sequences. Each also had a trap sampling for 4½ hours. Six such sequences were set up at random, 3 each on 17 and 18 May 1968 between 0800 and 1700 hours. The results are somewhat uneven but show that the fish are scarce in the traps out for short or long time intervals (table 1). The scarcity of fish in traps set for only 15 minutes is thought to reflect fright reaction to the disturbance associated with setting and removing the trap. (Another series at downstream diurnal where the traps could be removed and returned with less disturbance did not show this response.) The reduction of fish per trap hour in traps set for more than 2 hours indicates that an appreciable fraction of the fish in the trap were escaping. The data on the 4½ hour traps is likely to be more valid

for twice as many samples were available and the 9 hour traps were, of necessity, at stations not sampled for the shorter times on the same day. It seems likely, therefore, that trap intervals much greater than 2 hours will not greatly increase the number of fish per trap.

Fish Size

There is an obvious association of fish size with number of fish obtained in a trap. For example, a series of 25 traps on 5 October 1968 from downstream diurnal (fig. 3) had each of the 7 traps with fewer than 20 fish with an average fish size of less than 20.4 mm (total average of the 7 was 19.3 mm) whereas the entire sample averaged more than 20.7 mm (fig. 6). The positive association between size and number of fish in a trap could be a function of small fish being driven out of traps by large fish or small fish entering traps more quickly than large fish (sampling association), or the fish in optimal localities being larger than those in suboptimal habitats (sampling environmental size differences). The 25 individual trap captures indicate that the last-mentioned alternative probably accounts for most or all of the variation. The traps were placed in 5 spots for 3 hours and 45 minutes. Each station had one trap removed after 15, 30, 45, 60, and 75 minutes, and the trap for each time interval was removed first, second, etc. at each station. The average sizes for the 15-75 minute traps were 20.6, 20.9, 20.8, 21.0, and 20.4 mm, respectively, indicating little time-size correlation (table 2). The average sizes of the fish in the first traps lifted were 21.0 and those of both of the last two time intervals were 20.4 mm, indicating that big fish were removed early and that smaller ones from nearby areas were captured subsequently. This was confirmed by the association of fish size with capture station in which the traps from station 5 had twice the fish of any other station, and the fish had an average size more than 1 mm greater than the overall average. If adjustments are made for these 3 factors, the average size differences are virtually eliminated, the size-number association is less than 0.13 mm, and one trap with 16 fish has the largest average-sized fish. It is apparent, therefore, that the size of the fish in the trap reflects the size of the fish available to be captured and that large fish in the trap usually means that many fish are in the trap.

Time of Capture

The clock interval during which the traps are in the water may affect the number of fish in the various traps. Diel trap capture rates were studied on 4 different occasions. All show the same pattern of many fish captured during daylight intervals and few present at night. Two sampling intervals, 8-9

Table. 1. Captures in traps set different time periods on north shore of Clear Creek below dam.

TIME (MINUTES)	TOTAL FISH	FISH PER TRAP HOUR	FISH PER TRAP	FISH PER SUBSEQUENT TRAP HOUR	
15	156	13.0	3.3	13.0	
30	320	13.3	6.7	13.7	
45	616	17.1	12.8	24.7	16.30
60	898	18.3	18.7	23.5	
75	1004	16.7	20.9	8.8	
90	1176	16.3	24.5	14.3	
105	1369	16.3	28.5	16.1	19.95
120	1856	19.3	38.7	40.6	
270	450	8.3	37.5	0	
540	615	11.4	102.5	14.4	7.2

Table 2. Average sizes of fish obtained from the same area at different times, sequences, and places.

TIMES		PLACES		SEQUENCES	
15 min.	20.6±0.38	Station 1	20.9±0.35	traps removed first	21.0±0.31
30 min.	20.9±0.36	Station 2	19.4±0.26	second	20.5±0.21
45 min.	20.8±0.23	Station 3	21.1±0.26	third	21.4±0.27
60 min.	21.0±0.26	Station 4	19.1±0.21	fourth	20.4±0.28
75 min.	20.4±0.21	Station 5	21.9±0.22	last	20.4±0.29

Table 3. *Gambusia* captures at various clock hours in summer at Clear Creek. See text for locations and dates.

DOWNSTREAM DIURNAL

TIME (hrs.)	FISH/TRAP	MM/TRAP	AV. SIZE
0000 - 0200	1.54	32.3	20.93
0200 - 0400	1.54	32.0	20.76
0400 - 0600	1.23	24.9	20.25
0600 - 0800	22.79	520.6	22.84
0800 - 1000	64.79	1448.8	22.36
1000 - 1200	91.15	2071.7	22.73
1200 - 1400	56.27	1241.6	22.07
1400 - 1600	39.02	833.0	21.35
1600 - 1800	29.98	592.9	21.19
1800 - 2000	36.63	839.7	22.93
2000 - 2200	8.48	194.9	22.99
2200 - 2400	1.69	34.1	20.22

Table 4. *Gambusia* captures at various clock hours in summer at Clear Creek. See Fig. 3 for location and text for dates.

UPSTREAM DIURNAL

TIME (hrs.)	FISH/TRAP	MM/TRAP	AV/SIZE
0000 - 0200	0.33	7.2	21.75
0200 - 0400	0.38	7.9	21.11
0400 - 0600	0.25	5.4	21.67
0600 - 0800	20.08	425.5	21.19
0800 - 1000	11.75	252.6	21.50
1000 - 1200	22.12	450.9	20.38
1200 - 1400	38.25	825.7	21.59
1400 - 1600	11.92	230.6	19.35
1600 - 1800	10.67	208.9	19.59
1800 - 2000	13.46	289.4	21.50
2000 - 2200	4.29	93.2	21.71
2200 - 2400	0.46	8.6	18.73
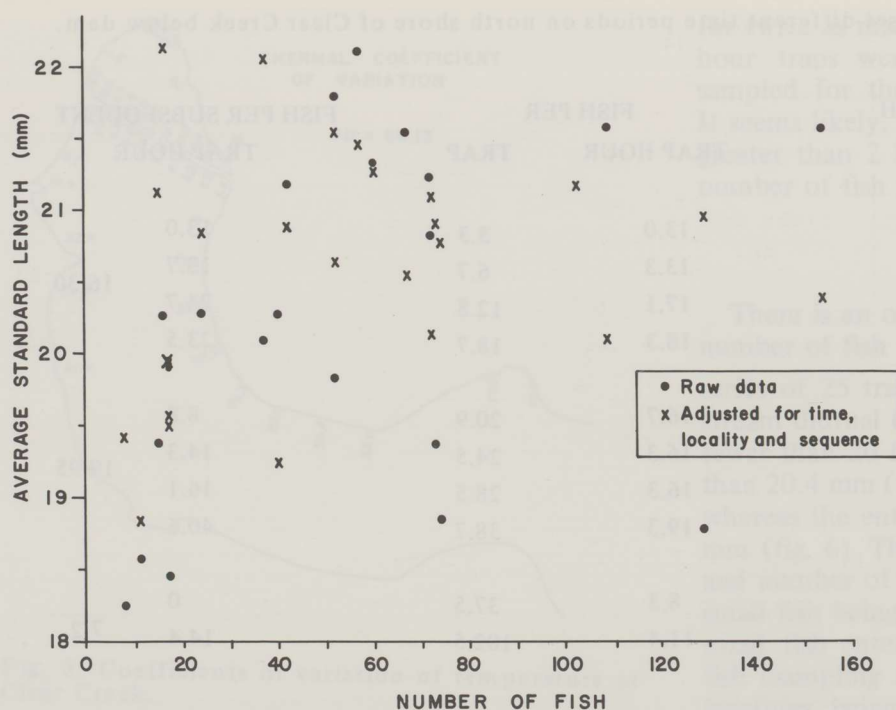
Fig. 6. Average size of 25 samples of fish obtained from downstream diurnal 5 October, 1968.

The scatter plot shows AVERAGE STANDARD LENGTH (mm) on the y-axis (18 to 22) versus NUMBER OF FISH on the x-axis (0 to 160), with legend: • Raw data; x Adjusted for time, locality and sequence.

March and 16-17 May 1969, are not discussed because few fish were obtained on the second day. This would permit a hypothesis that some of the low fish capture rate occurring at night might reflect a reduced population present caused by trapping effort the previous day. Nevertheless, more fish were obtained in the morning than during the previous night. At the other two intervals, subsequent daylight capture rates greatly exceeded those during the preceding night and were equivalent to or exceeded the capture rates during the same intervals the previous day.

Twenty-four traps set between stations H and downstream diurnal (fig. 3) and between K and M and removed at 2-hour intervals from 1000 hours 28 July 1969, and 1400 hours 29 July 1969, and six traps set at downstream diurnal between 1000 hours 4 August 1968, and 0800 hours 6 August 1968, contained 22,290 fish (table 3). More than 10 fish were taken per trap each 2-hour interval between 0800 and 2000 hours (daylight) and fewer than 2 fish at comparable time spans between 2200 and 0600 hours. To avoid individual station variations, the capture rates were averaged
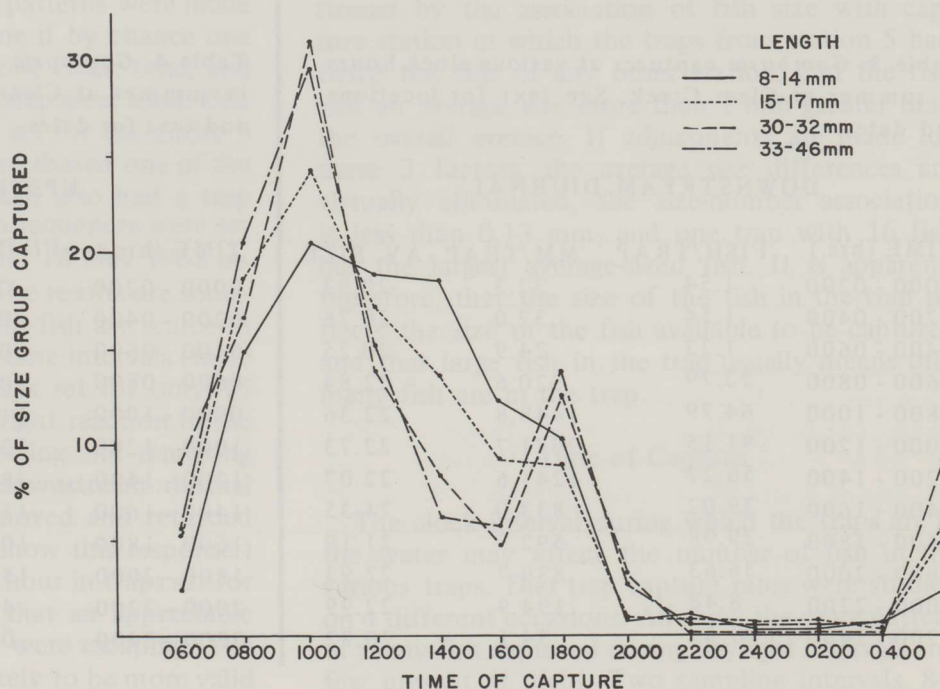
Fig. 7. Capture rates for four size classes of *Gambusia affinis* during different clock hours at Clear Creek.

The line graph shows % OF SIZE GROUP CAPTURED (y-axis, up to 30) versus TIME OF CAPTURE (x-axis, 0600 to 0400) with LENGTH legend: 8-14 mm, 15-17 mm, 30-32 mm, 33-46 mm.

8

Fig. 8. Association of hy-
brid index code with
relative activity of adult
fish in upstream and
downstream diurnal. The
capture ratios (down-
stream-upstream) are the
relative trapping rates
during daylight hours
for fish over 30 mm. The
hybrid index code is the
ratio of the hybrid index
in the individual upstream
diurnal sample with that
of the total sample from
that locality. The hybrid
index is obtained by
methods reported by
Hubbs (1959). The
numbers beside the plots
are the number of adults
used to calculate the
index average. The fig-
ures on the far right are
for 1000-1200 hrs.
(downstream activity
peak) and on the far left
for 0600-0800 hrs. (up-
stream activity peak).

AVERAGE HYBRID INDEX (y-axis, 90 to 105)
CAPTURE RATIO (x-axis, 0 to 25)

Data points: •14, •6, •25, •89, •30, •40, 146•, •29, 40•, •6, •4, •6, •23, •13, •27, •88

for both trap series before combining the data.

These data also show that the fish captured at night average smaller than those captured during daylight intervals. Because of the large sample size, it was possible to plot capture rates of various-sized fish. If the total captures for 8 size classes are divided into the captures between 2200 and 0600 hours, the percentages are:

MM	Percent
8-14	=	2.7
15-17	=	2.9
18-20	=	2.2
21-23	=	1.4
24-26	=	0.9
27-29	=	0.8
30-32	=	0.6
33-36	=	1.5

The last size class was born the preceding year; therefore, there is a distinct decrease of night capture rate with size for fish that have not lived through one winter. Because most of the 8-14 mm captures were 13 or 14 mm, it is possible the small fish sample is distorted and that an adequate sample of 7-9 mm fish would show nearly equivalent capture rates at all time intervals. The daytime capture rates have a distinct peak at about 1000 hours and a less pronounced one at dusk (1800-2000). At both times the average size of the fish captured is larger than that of fish obtained during intervening daylight intervals. The size related variations in activity clearly have a dusk activity peak in large fish

but only an indication of the same phenomenon in small fish (fig. 7). The intermediate size classes have intermediate dusk captu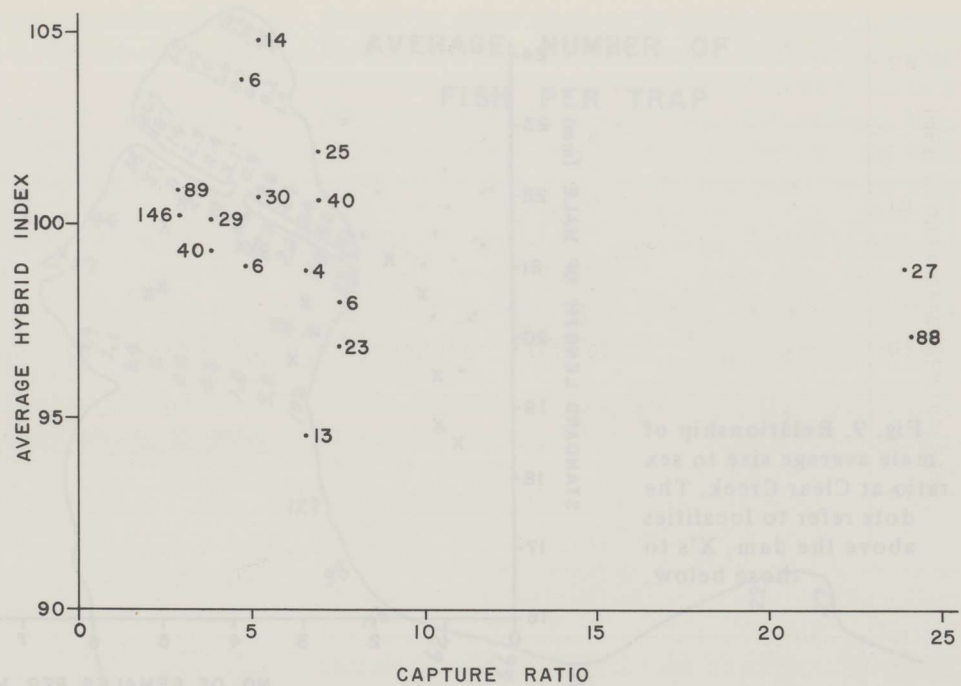re rate peaks. The size related differences in activity cycles are similar to sample differences reported by Deacon and Wilson (1967) and may account for some of their sample differences. Rensing (1965) reported that many animals have more pronounced diel rhythms when mature than when young.

A similar series of 6 traps set at upstream diurnal (fig. 3) from 1000 hours 28 July to 1400 hours 29 July 1969, and 1800 hours 4 August to 0800 hours 6 August 1968, contained many more fish at day than at night (table 4). Similarly, an evening activity peak can be noted and the large average size of evening-captured fish shows that this peak is size-related like that of samples collected at downstream diurnal. The daytime rates were some-what dissimilar between the two series. Relatively few fish were obtained at upstream diurnal during the peak capture interval, 1000-1200 hours, of downstream diurnal. The difference is not based on locality because the hybrid index code at upstream is low (*affinis*-like) when downstream diurnal is relatively active and high when upstream diurnal is active (fig. 8).

Each month (October to October) traps were set the night following the regular sampling interval in B, E, F, G, K, and L. The night captures were typically of few and small fish. If the overnight traps were not removed until after daylight, captures were increased, showing that the dark quiet periods are reasonably well maintained during all seasons.

In contrast to Maglio and Rosen's (1969) observations on Illinois *G. affinis* in a New York pond, my nighttime trap captures in or over deep water were not as great as those at the same spots during

Fig. 9. Relationship of male average size to sex ratio at Clear Creek. The dots refer to localities above the dam, X's to those below.
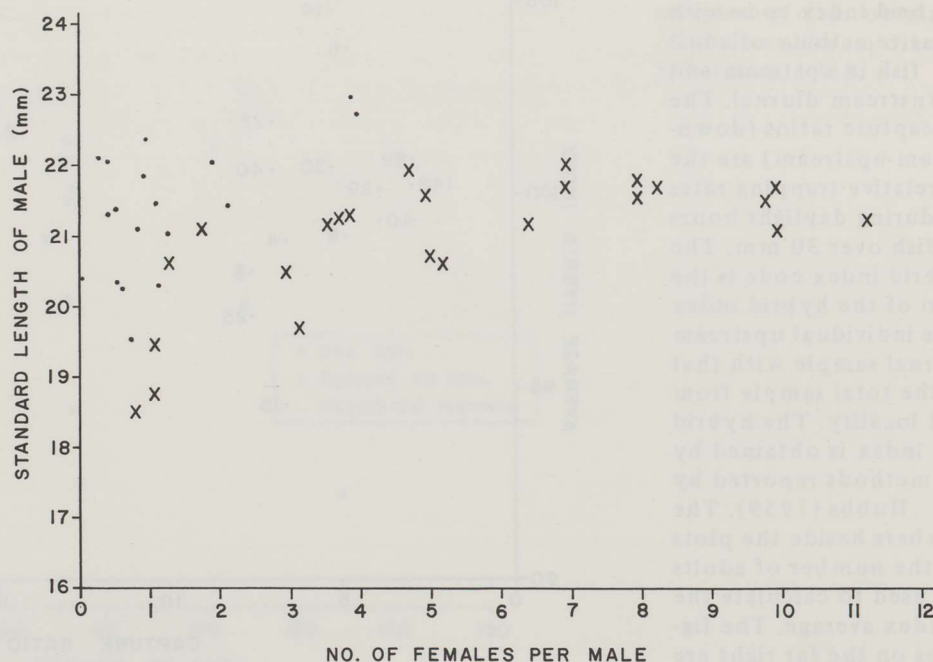
daylight hours. The difference may be due to stock or sample variation or, more likely, at night inactive *Gambusia* sink to the bottom at the site they occupied at dusk. Their report of a midday activity peak (1300) is concordant with a *G. affinis* peak between 1000 and 1200 hours, especially in the absence of their observations between 0900 and 1300 hours. Furthermore, their report of *Gambusia* following microhabitat thermal changes is in accord with observations during the March diurnal on which date one station set near a small spring obtained many fish early in the morning but few in the afternoon.

Sex Ratio

Most analyses of poeciliid natural populations conclude that more mature females are present than mature males. Although it is difficult to be certain of the degree of maturity of individuals obtained in non-reproductive seasons, the samples obtained here usually have more females than males. Male maturity is considered to have been attained if the gonopodial tip is clear and translucent; females are considered mature when they have reached 25 mm standard length. Such allocation favors males because more than half of the 20 mm females were mature in July 1969. Arbitrary categorization permits comparisons among stations and seasons. Other criteria used to distinguish the sex of young *Gambusia,* fine structure of the male anal fin (Turner, 1941), the thickened upper pectoral rays of males (Rosen and Bailey, 1963), and, of course, gonadal anatomy, were not used because of difficulties associated with processing large samples. Moreover, use of immature individuals

would overlook environmental modification of genetic sex inheritance (Haskins *et al.,* 1961; Harrington, 1967), distortions that would be best reduced in immature fish by use of time-consuming microscopic examination of gonads.

The stations above the dam averaged 140±59 females and 74±17 males, the single trap stations below the dam averaged 86±37 females and 19±5 males, and the triple trap stations (F-R) below the dam averaged 977±133 females and 147±18 males. In each area more females than males were obtained, despite the distortion due to allocating many mature females as immature. Within each of the above three categories the females were not only more numerous than males, but their numbers varied more. For each situation the coefficient of variation (212♀ vs 114♂, 198♀ vs 120♂, and 49♀ vs 45♂) was greater for females than males, indicating that males were more evenly distributed spatially in the environment than females. Baird (1968) has shown similar sexually different distribution patterns in *Mollienesia latipinna* and has shown that the males are much more territorial than females. If one assumes that the known male aggression in *Gambusia* (McAlister, 1958) has a field effect similar to that in *Mollienesia,* dominant males would force subordinate males into unfavorable environments. Because McAlister (1958) showed that dominance is correlated with size in *Gambusia,* male size was plotted against sex ratio (fig. 9). The tendency of small males to have fewer mature females available clearly demonstrates the selective advantage of size. Because *Gambusia* mature males do not grow, a male that matures early is likely to be so small that he would be behaviorally excluded from the gene pool, and one that has his maturity unduly delayed

AVERAGE NUMBER OF
FISH PER TRAP

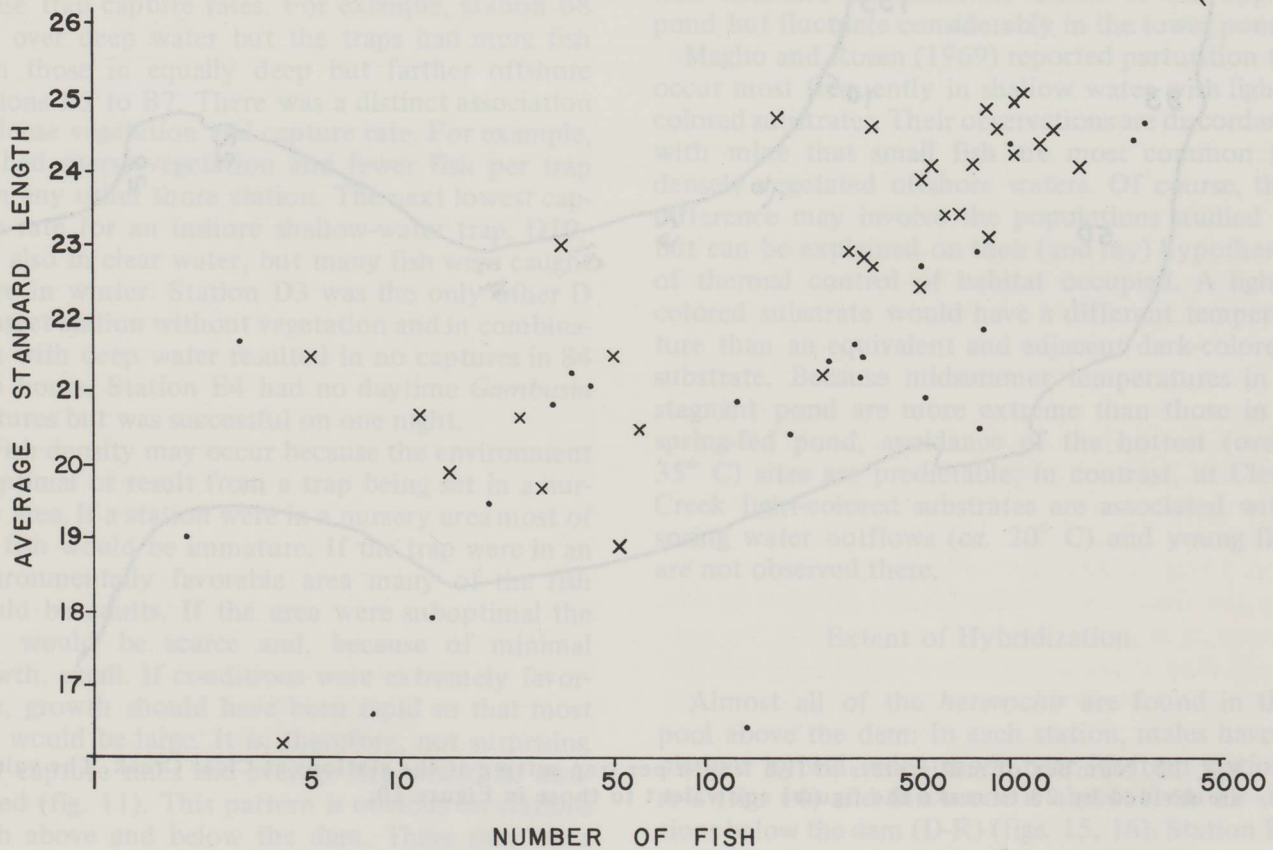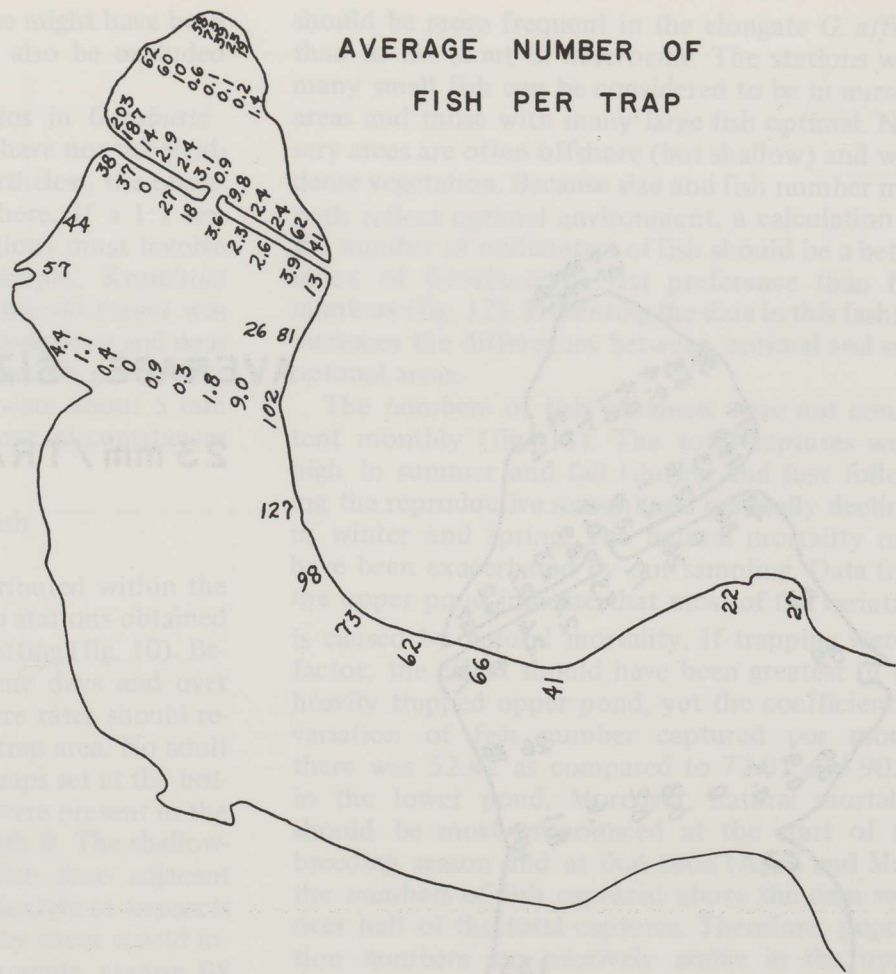23 mm / SL

AVERAGE STANDARD LENGTH

NUMBER OF FISH

Fig. 11. Relationship of average size to number of fish per trap at Clear Creek. Dots signify above dam station, X's below damsites.
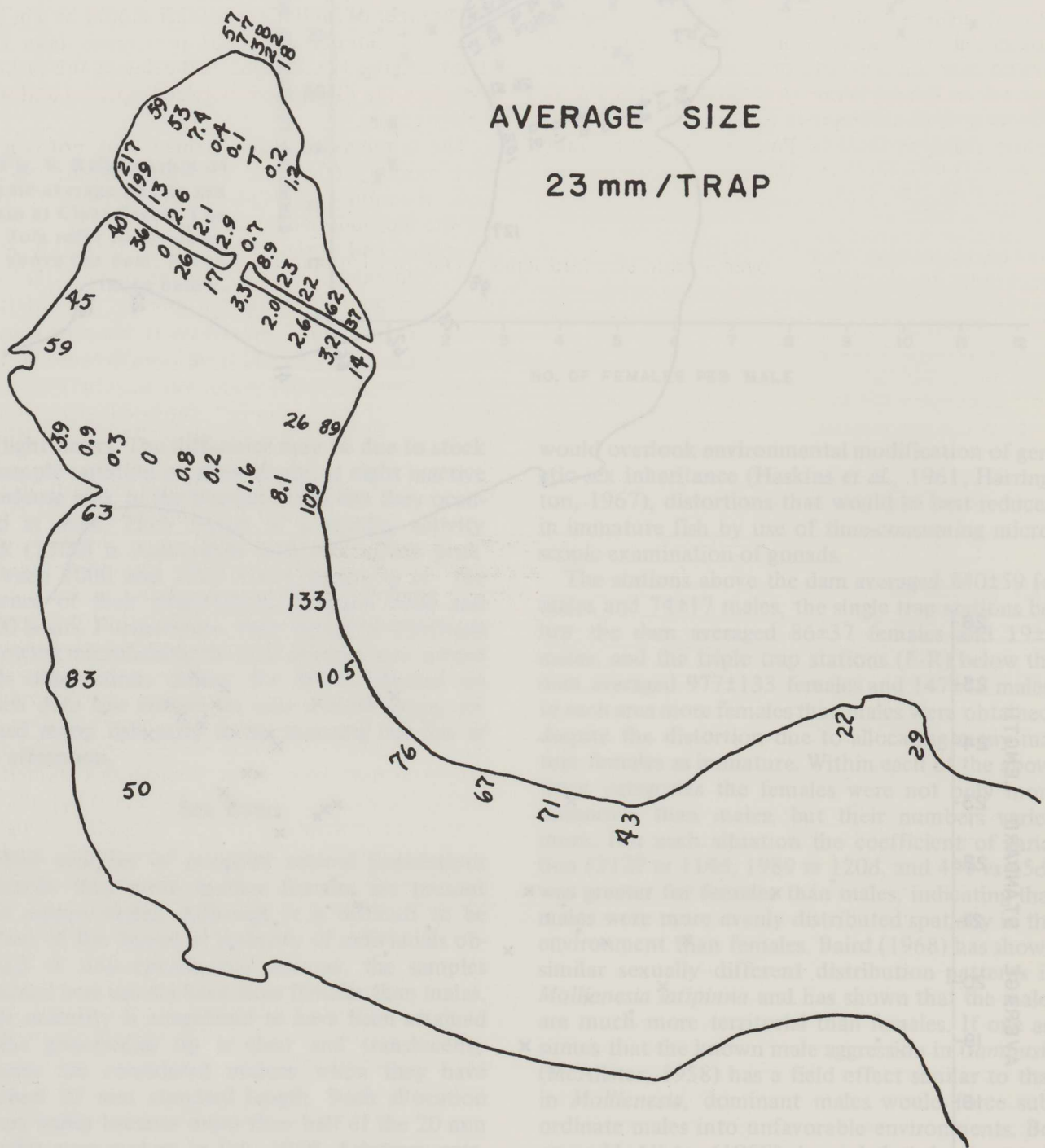
11

Fig. 12. Number of millimeters of fish length per trap setting at the stations at Clear Creek. The values are divided by 23 to make the figures equivalent to those in Figure 10.

and obtains subsequent dominance might have been consumed before maturity and also be excluded from the gene pool.

Previous analyses of sex ratios in *Gambusia* (Krumholz, 1963, and citations) have not subdivided ponds as was done here. Nevertheless, the observations support those reported here. If a 1:1 sex ratio at birth is assumed, deviations must involve differential mortality. For example, Krumholz showed that the sex ratio of *Gambusia manni* was near 1:1 in Daniels' Pond (low predation) and near 2♀♀:1♂ in Caloo Mangrove (many fish predators). Mature males in Daniels' Pond were about 5 mm longer than those in Caloo Mangrove, circumstances parallel to those reported here.

Abundance of Fish

Gambusia are not evenly distributed within the aquatic habitat. The standard trap stations obtained up to 203 fish per 6-hour trap setting (fig. 10). Because the traps were set the same days and over comparable time intervals, capture rates should reflect population densities in the trap area. No adult *Gambusia* were obtained in the traps set at the bottom, and usually more juveniles were present in the floating traps than the one beneath it. The shallow-water stations averaged more fish than adjacent floating traps. This was most noticeable at transects B and E. The presence of a nearby shore would increase trap capture rates. For example, station B8 was over deep water but the traps had more fish than those in equally deep but farther offshore stations B5 to B7. There was a distinct association of dense vegetation and capture rate. For example, E1 had sparse vegetation and fewer fish per trap than any other shore station. The next lowest capture rate for an inshore shallow-water trap, D10, was also in clear water, but many fish were caught there in winter. Station D3 was the only other D transect station without vegetation and in combination with deep water resulted in no captures in 84 trap hours. Station E4 had no daytime *Gambusia* captures but was successful on one night.

Fish density may occur because the environment is optimal or result from a trap being set in a nursery area. If a station were in a nursery area most of the fish would be immature. If the trap were in an environmentally favorable area many of the fish would be adults. If the area were suboptimal the fish would be scarce and, because of minimal growth, small. If conditions were extremely favorable, growth should have been rapid so that most fish would be large. It is, therefore, not surprising that capture rates and average size would be associated (fig. 11). This pattern is obvious on stations both above and below the dam. There may be a slightly greater relative size of fish from stations below the dam than from comparable densities above the dam. This is thought to be due to escape of small fish through holes in trap funnels that

should be more frequent in the elongate *G. affinis* than in the stout *G. heterochir*. The stations with many small fish can be considered to be in nursery areas and those with many·large fish optimal. Nursery areas are often offshore (but shallow) and with dense vegetation. Because size and fish number may both reflect optimal environment, a calculation of the number of millimeters of fish should be a better index of *Gambusia* habitat preference than fish numbers (fig. 12). Presenting the data in this fashion increases the differences between optimal and suboptimal areas.

The numbers of fish obtained were not consistent monthly (fig. 13). The total captures were high in summer and fall (during and just following the reproductive season) and gradually declined in winter and spring. The natural mortality may have been exacerbated by our sampling. Data from the upper pond indicate that most of the variation is caused by natural mortality. If trapping were a factor, the effect should have been greatest in the heavily trapped upper pond, yet the coefficient of variation of fish number captured per month there was 52.42 as compared to 77.07 and 90.66 in the lower pond. Moreover, natural mortality should be most pronounced at the start of the breeding season and at that time (April and May) the numbers of fish captured above the dam were over half of the total captures. Therefore, population numbers are relatively stable in the upper pond but fluctuate considerably in the lower pond.

Maglio and Rosen (1969) reported parturition to occur most frequently in shallow water with light-colored substrates. Their observations are discordant with mine that small fish are most common in densely vegetated offshore waters. Of course, the difference may involve the populations studied — but can be explained on their (and my) hypothesis of thermal control of habitat occupied. A light-colored substrate would have a different temperature than an equivalent and adjacent dark-colored substrate. Because midsummer temperatures in a stagnant pond are more extreme than those in a spring-fed pond, avoidance of the hottest (over 35° C) sites are predictable; in contrast, at Clear Creek light-colored substrates are associated with spring water outflows (*ca.* 20° C) and young fish are not observed there.

Extent of Hybridization

Almost all of the *heterochir* are found in the pool above the dam. In each station, males have a distinct hybrid index mode near 90 from stations A-C (fig. 14) and this mode is absent from all stations below the dam (D-R) (figs. 15, 16). Station B2 seems to have the highest *heterochir* frequency. Above the dam, *affinis* are most abundant at station C12. The frequency of hybrid males varies but seems to be associated with frequency of *affinis*
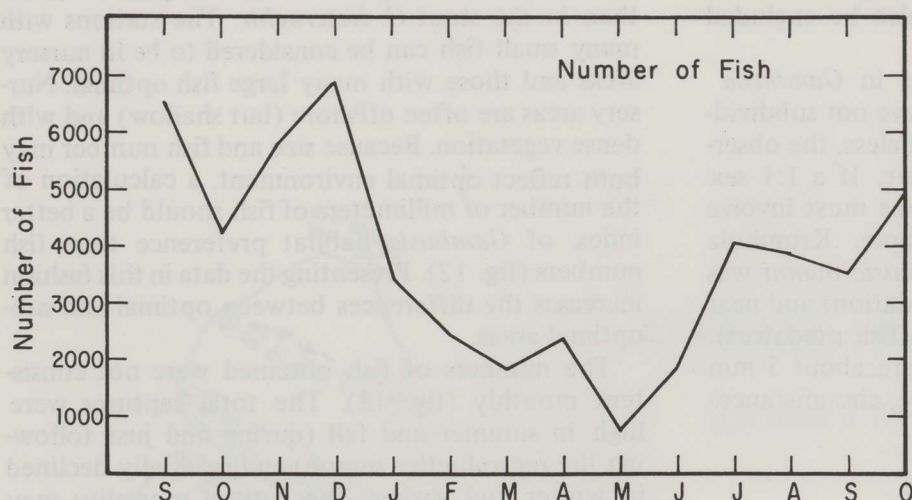
Number of Fish

males. In the upper pool, most of the hybrids and *affinis* seem to be concentrated along the east end of the dam in stations C10-12.

None of the males obtained below the dam can be considered to be pure *heterochir* (figs. 15, 16). The highest indices are in the low 80's and a male with a code of 85 might questionably be *heterochir*. The frequency of putative hybrid males is low below the dam, and the stations near the dam (D) have more than do those distant from springs (L-P) (fig. 16).

The pattern of the females is similar to that of males. Almost all of the *heterochir* are above the dam. In each plot from stations A-C (fig. 17), the females have a mode about 100 and this mode is absent from all stations below the dam (D-R) (figs. 18, 19). Stations B2, C1, and C2 seem to have the highest *heterochir* frequencies. Above the dam, *affinis* females are most abundant at station C12. Because females are more similar than males, hybrids are difficult to ascertain with assurance but seem to occur at all localities and not to be as dependent as males on *affinis* frequency.

None of the plots from below the dam has a mode in the 95-105 range (*heterochir*) and many plots have no specimens in this range (figs. 18, 19). In contrast to the males, several stations (notably D4 and E10) have females that appear to be pure *heterochir*. Therefore, *heterochir* females seem more likely to be collected in the samples from below the dam. The *heterochir* and putative hybrid frequency is much greater in stations near the dam (D-G) than in those at a distance (H-R). The difference is more striking than in male plots. In contrast to the males, female hybrids are infrequent. For example, station K had no putative hybrids in more than 2,000 females while 10 of the 364 males captured were hybrids.

Hereafter, females with index codes of 80 or less are considered *affinis* and those above 95 *heterochir*. Hybrids are considered to be those with

index values of 86 to 90 and those with intermediate values are called backcross hybrids. Similarly, males are allocated according to the following code values: up to 58, *affinis;* 59-65, backcrosses; 66-75, hybrids; 76-82, backcrosses; and 83 or higher, *heterochir*. Individual allocations may be in error but most fish in a category are more similar to others in that category than to those in other (especially non-adjacent) categories.

Changes in Time

The general distribution of parental phenotypes and putative hybrids is similar in the 1956-57 and 1967-68 collections. Because *Gambusia* seldom live more than one year at Clear Creek (and elsewhere; Krumholz, 1948) and may become mature in about three months (see below), 10-20 generations have elapsed between samples. The temporal changes are best shown by superimposed plots. Although individual station contrasts may show detailed population changes, the patterns are more critical; therefore, each sample interval has been pooled into three groupings: above dam, consistent temperature below dam, and fluctuating temperature below dam. In each study, an *affinis* mode is apparent in each grouping, but *heterochir* modes are obvious only above the dam (figs. 20-25). The female *affinis* modes are at 77 below the dam and seem to be 78 above the dam. The female *heterochir* mode was about 98 in 1956-57 and 100 in 1967-68. The difference suggests reinforcement which is contradicted by the male plots. The male *affinis* below the dam have modes of 53-55 with a tendency for the 1967-68 data to have lower figures than the 1956-57 plots; however, the *heterochir* plots have modes of 90 (1967-68) and 93 (1956-57). If one assumes the differences to be three code units, the males are 3/35 closer together after 10 years, but the females are 2/23 farther apart. Although it is possible that females have re-
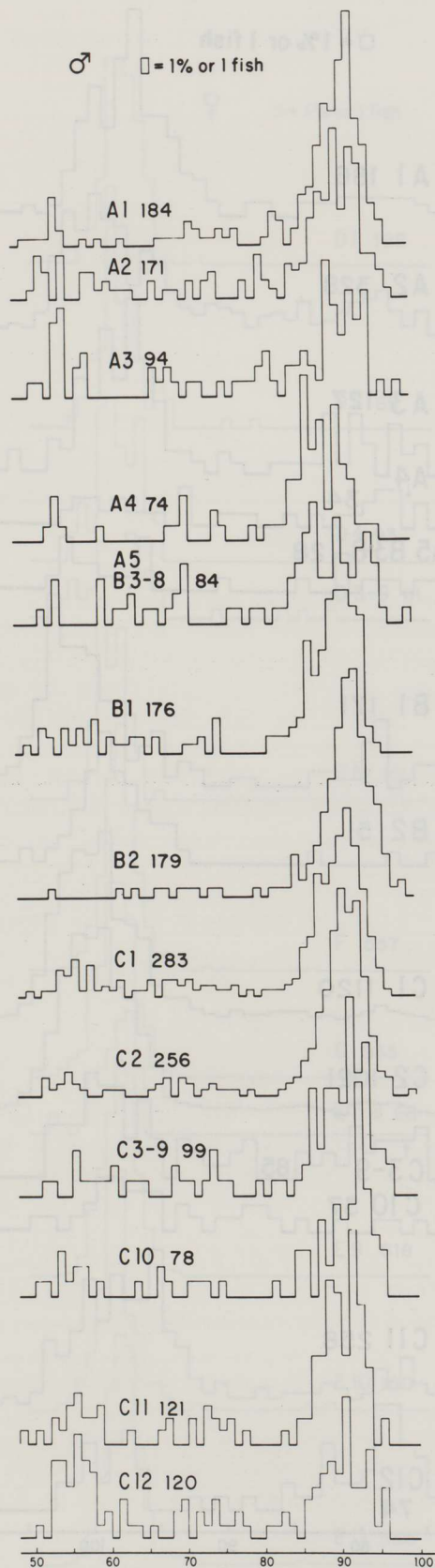
Fig. 14. Relative frequency of hybrid index values of males taken from stations above the dam at Clear Creek. The methods for obtaining code values are the same as reported in Hubbs (1959). Typical *G. affinis* would have modes near 54 and *G. heterochir*, near 90.

Fig. 15. Relative frequency of hybrid index values of males taken from stations below the dam in consistent temperature regimes. The methods for obtaining code values are the same as reported in Hubbs (1959). Typical *G. affinis* would have modes near 54 and *G. heterochir*, near 90.

♂ □ = 1% or 1 fish

Number of Fi... ♀ □ = 1% or 1 fish

H 195
I 163
J 133
K 364
L 173
M 126
N 152
O 135
P 9
Q 51
R 54

A1 186
A2 328
A3 127
A4 34
A5 B3-8 28
B1 171
B2 51
C1 1120
C2 1021
C3-9 85
C10 37
C11 258
C12 74

Fig. 16. Relative frequencies of hybrid index values of males taken from stations below the dam in thermally variable regimes. The methods for obtaining code values are the same as reported in Hubbs (1959). Typical *G. affinis* would have modes near 54 and *G. heterochir*, near 90.

Fig. 17. Relative frequency of hybrid index values of females taken from stations above the dam at Clear Creek. The methods for obtaining code values are the same as reported in Hubbs (1959). Typical *G. affinis* would have modes near 77 and *G. heterochir*, near 100.

16

♀ □ = 1% or 1 fish

D1 198

D2 89

D4 93

D5-9 61

D10 80

F 557

G 955

E1-8 58

E9 618

E10 150

E11 558

70 80 90 100 110

♀ □ = 1% or 1 fish

H 852

I 1276

J 691

K 2028

L 1715

M 1245

N 1162

O 1160

P 641

Q 235

R 499

70 80 90 100 110

Fig. 18. Relative frequency of hybrid index values of females taken from stations below the dam in consistent temperature regimes. The methods for obtaining code values are the same as reported in Hubbs (1959). Typical *G. affinis* would have modes near 77 and *G. heterochir*, near 100.

Fig. 19. Relative frequencies of hybrid index values of females taken from stations below the dam in thermally variable regimes. The methods for obtaining code values are the same as reported in Hubbs (1959). Typical *G. affinis* would have modes near 77 and *G. heterochir*, near 100.
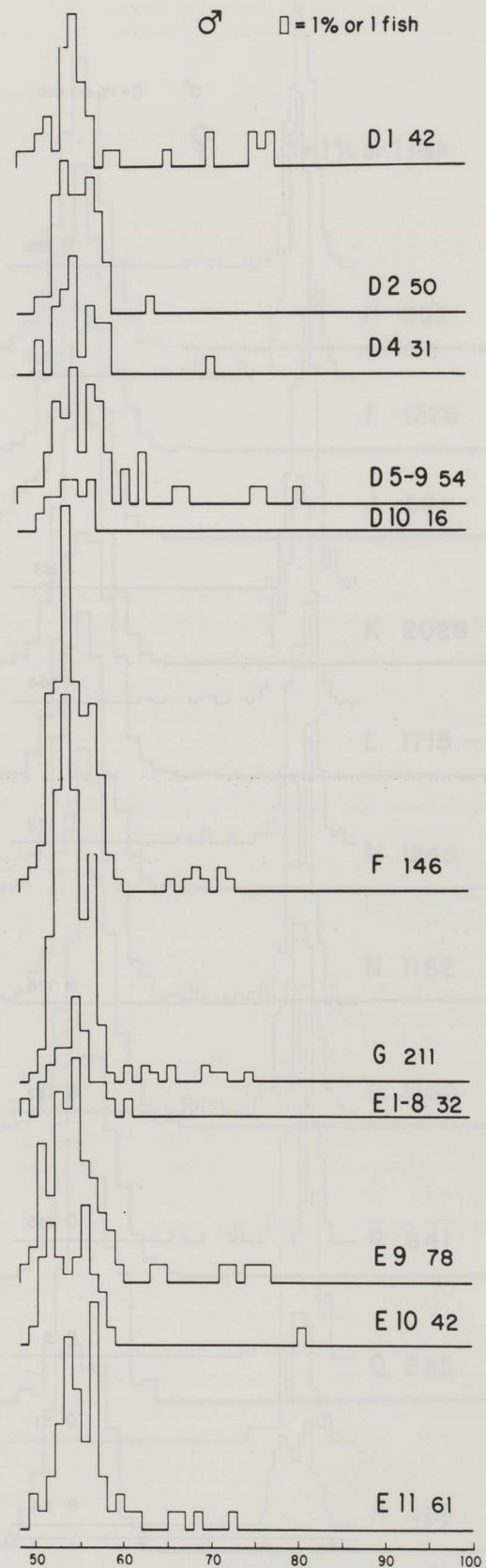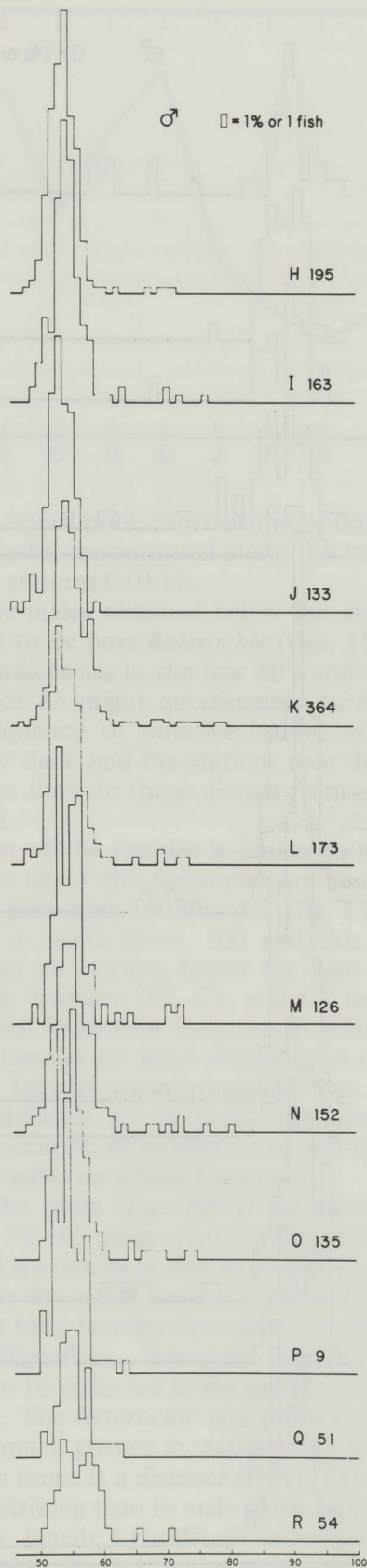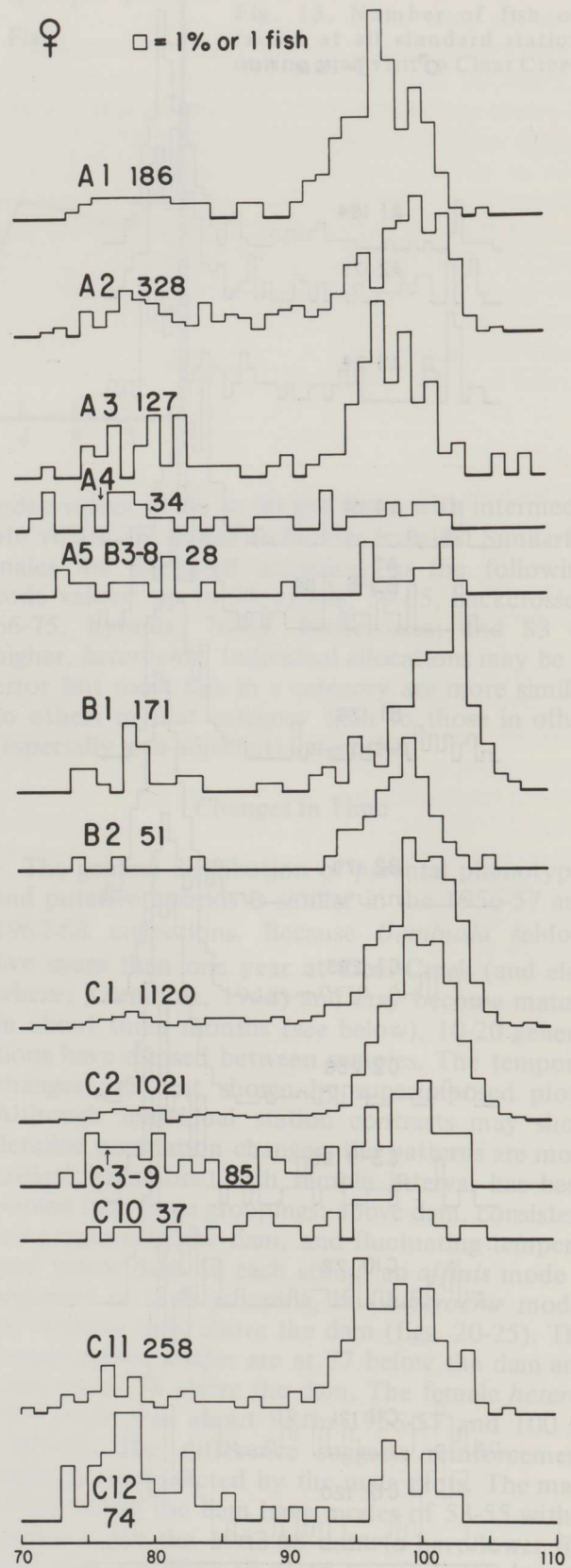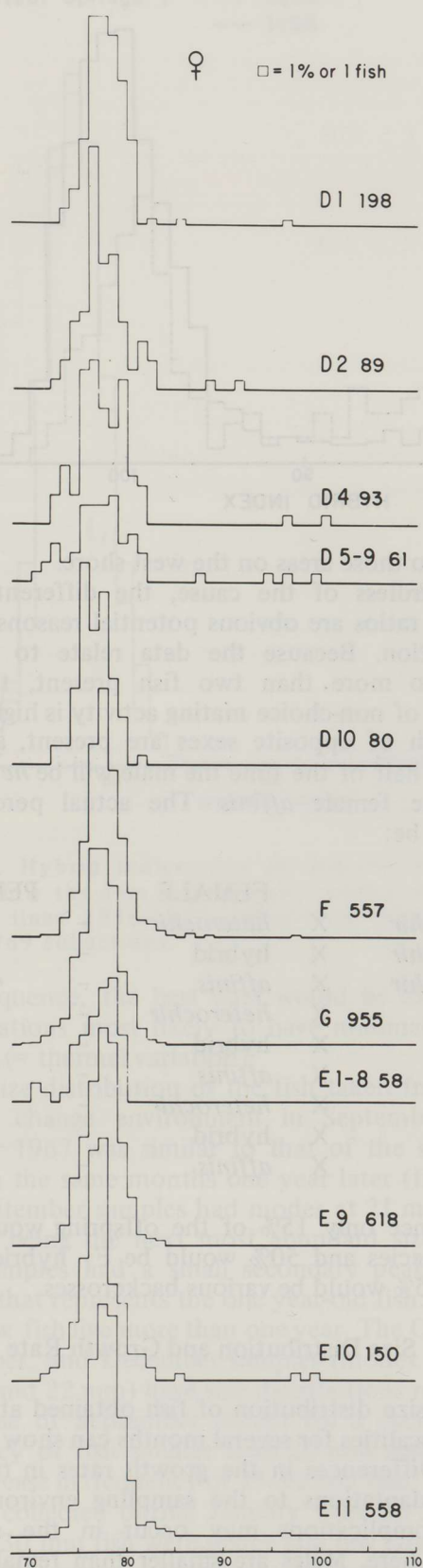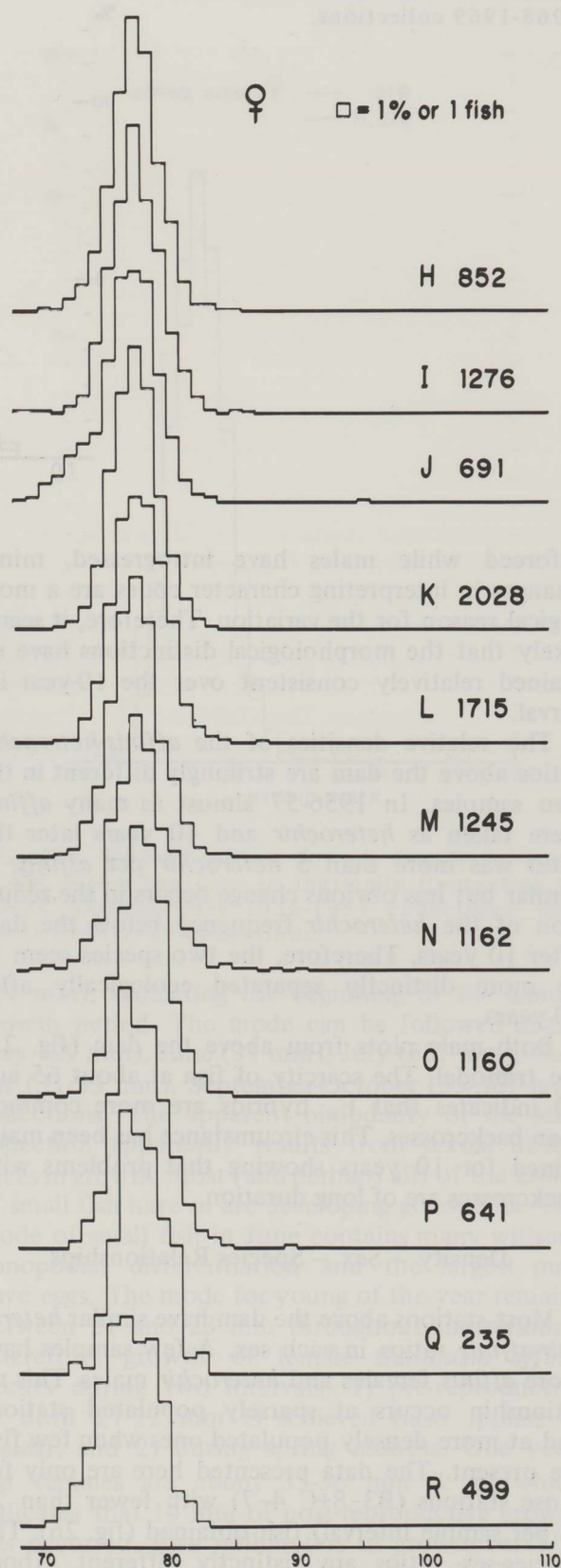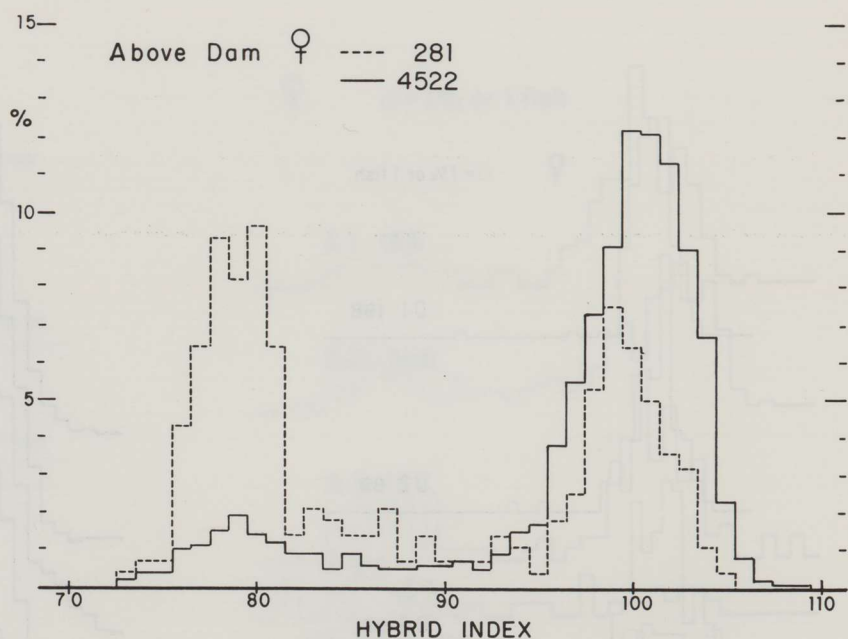
17

Fig. 20. Hybrid indices for all fe-
males obtained from above the
dam at Clear Creek. Dashed lines
1956-1957 collections. Solid lines
1968-1969 collections.

Above Dam ♀ ---- 281
— 4522

HYBRID INDEX

inforced while males have introgressed, minor
changes in interpreting character codes are a more
logical reason for the variation. Therefore, it seems
likely that the morphological distinctions have re-
mained relatively consistent over the 10-year in-
terval.

The relative densities of the *affinis-heterochir*
ratios above the dam are strikingly different in the
two samples. In 1956-57 almost as many *affinis*
were taken as *heterochir* and 10 years later the
ratio was more than 5 *heterochir* per *affinis*. A
similar but less obvious change occurs in the reduc-
tion of the *heterochir* frequency below the dam
after 10 years. Therefore, the two species seem to
be more distinctly separated ecologically after
10 years.

Both male plots from above the dam (fig. 23)
are trimodal. The scarcity of fish at about 65 and
80 indicates that F_1 hybrids are more common
than backcrosses. This circumstance has been main-
tained for 10 years showing that problems with
backcrosses are of long duration.

Density — Sex — Species Relationships

Most stations above the dam have similar *hetero-
chir-affinis* ratios in each sex. A few samples have
more *affinis* females and *heterochir* males. This re-
lationship occurs at sparsely populated stations
and at more densely populated ones when few fish
are present. The data presented here are only for
those stations (B3-8+C 4-7) with fewer than 28
(2 per sample interval) fish obtained (fig. 26). The
species-sex ratios are distinctly different. About
70% of the males are *heterochir* (7% *affinis*) and
70% of the females *affinis* (15% *heterochir*). The
reversal is thought to result from the presence of
omega *heterochir* males and *affinis* females migrat-
ing from the dense populations adjacent to the dam

break to those areas on the west shore.

Regardless of the cause, the differential sex-
species ratios are obvious potential reasons for hy-
bridization. Because the data relate to samples
with no more than two fish present, the pos-
sibility of non-choice mating activity is high. When
two fish of opposite sexes are present, approxi-
mately half of the time the male will be *heterochir*
and the female *affinis*. The actual percentages
would be:

MALE		FEMALE		PERCENT
heterochir	X	*heterochir*	–	10
heterochir	X	hybrid	–	10
heterochir	X	*affinis*	–	49
hybrid	X	*heterochir*	–	3
hybrid	X	hybrid	–	3
hybrid	X	*affinis*	–	16
affinis	X	*heterochir*	–	1
affinis	X	hybrid	–	1
affinis	X	*affinis*	–	5

By chance only 15% of the offspring would be a
pure species and 50% would be F_1 hybrids. The
other 35% would be various backcrosses.

Size Distribution and Growth Rate

The size distribution of fish obtained at one or
more localities for several months can show growth
rates. Differences in the growth rates in turn can
show adaptations to the sampling environments.
Two complications may occur in the samples
studied here. Males are smaller than females, and
this difference could result in a size bimodality
based on sexual differences of equal-aged adults.
In addition, newborn fish are not easily allocated
to species and/or hybrids so that young size classes
may include distinctly different genetic stocks. As

18

Fig. 21. Hybrid indices for all females obtained from below the dam in consistent thermal stations. Dashed lines 1956-1957 collections. Solid lines 1968-1969 collections.

Fig. 22. Hybrid indices for all females obtained from below the dam in thermally fluctuating stations. Dashed lines 1956-1957 collections. Solid lines 1968-1969 collections.

a consequence, the best data would be expected from stations most likely to have minimal introgression (= thermal variation).

The size distribution of the fish taken from the thermal change environment in September and October 1967 was similar to that of the samples taken in the same months one year later (fig. 27). The September samples had modes at 21 mm with 22 mm being the next most abundant size class. Both samples had a small secondary peak about 35 mm that represents the one year-old fish. Therefore, few fish live more than one year. The October, November, and December samples (modes 21 and 22, 22 and 22 mm) have size distributions resembling those of September except for a decrease in frequency of fish smaller than 20 mm and a relative increase in frequency of 25-30 mm fish. Almost all fish collected during January were 20-24 mm. The 25-30 mm fish were scarce and few larger ones taken. The relative abundance of small fish continued in contrast to the scarcity of large fish during the February and March collections which had modal values of 23 and 22 mm respectively. The mode of the April collection was markedly larger

(24 mm), indicating the beginning of the annual growth period. The mode can be followed in the May (29 mm), June (31 mm), July (33? mm), August (31-33 mm), September-October (35-36? mm) collections. The apparent bimodality of the May collection apparently results from sexual differences in growth. Most (and perhaps all) of the group of small fish have or are developing gonopodia. The mode of small fish in June contains many without gonopodial differentiation and the largest may have eggs. The mode for young of the year remains between 21 and 23 mm throughout the summer. Therefore, growth of female *Gambusia affinis* occurs during two intervals: 1) pre-reproductive or until 21-22 mm is achieved (also applies to males); and 2) during spring months. One year-old females are about 35-36 mm S. L. which indicates that 14 mm of post-reproductive growth has occurred. Five millimeters of growth (or 1/3) seem to occur in April and another 1/3 in the two adjacent months. Some young-of-the-year females are sexually mature in early June and most over 22 mm in July have eggs or embryos. Because few females from the previous year sur-

19

Fig. 23. Hybrid indices for all males obtained from above the dam at Clear Creek. Dashed lines 1956-1957 collections. Solid lines 1968-1969 collections.
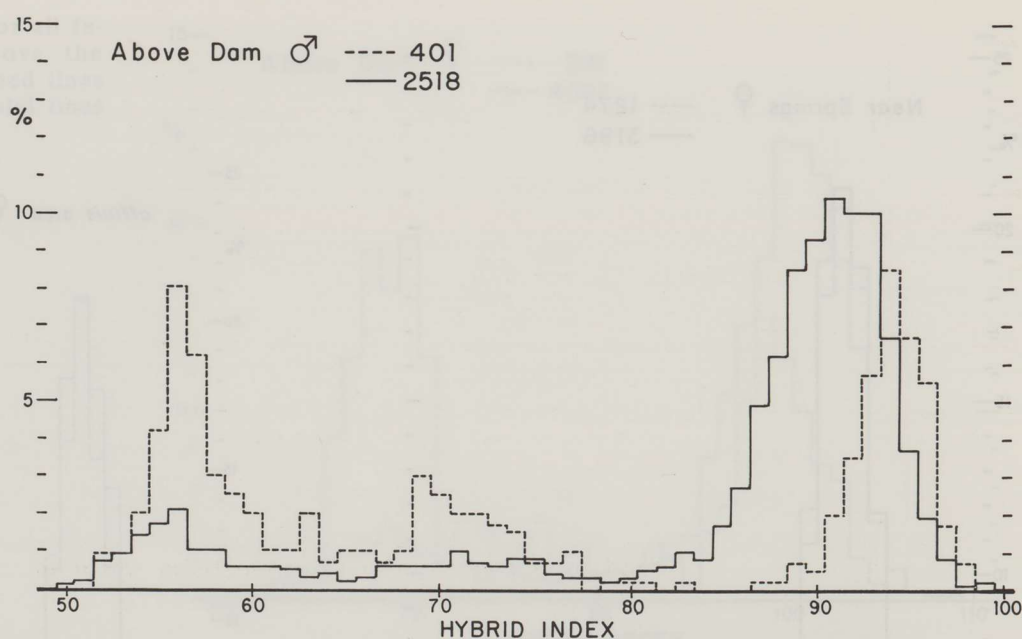
vive midsummer, most of the fall young are produced by females born that spring.

The size distribution of fish from consistent environments below the dam is similar to that of like fish from fluctuating environments, with a few exceptions (fig. 28). The yearling mode is not apparent in fall collections. The October collections have approximately the same number of small fish as the September samples; the decrease in frequency of small fish is delayed until November. The modal values are 21-22 mm in September through March samples, but the number of large fish is greater in winter than in fall. This is most pronounced in the January sample. The change in size distribution in the two environments indicates that the large fish (exclusively females) migrate from thermal change to habitats of thermal consistency in winter months only to return in the spring. The spring growth increase is demonstrated by modal size frequencies at 24 mm (April), 26 mm (May), and 28 mm (June and July), yet the increase during the "growth" months is 3-5 mm less than at the thermal change stations. Similarly, the pronounced peak of young fish at thermal change stations in June is difficult to demonstrate at thermally consistent samples, and the July mode of young-of-the-year is 20 rather than 22 mm. The reduced growth rate of fish from thermally consistent environments indicates that G. affinis is adapted to the thermal change environment. Although it is possible to hypothesize that the adaptation is to warm water, the most apparent differences are in April and May, months in which the average temperatures are comparable.

The size distribution of the Gambusia obtained from above the dam is rather consistent (fig. 29). The modes range between 20 and 23 mm. The only apparent bimodality occurs in the March 31 (30 mm) and May (26 mm) collections, and the secondary peaks are not associated with peaks in

adjacent months. Therefore, year classes do not show obvious size differentiation. The reasons are thought to be: 1) more or less level fecundity during the breeding season and 2) most of the newborn (heterochir) are larger than affinis and enter the trap-susceptible group earlier in the season.

Male Gambusia are known to cease growth upon sexual maturity (Krumholz, 1948 and citations); therefore, changes in male size frequencies would demonstrate migration or maturation of a new group of males. In winter most male affinis are larger than they are in summer (fig. 30). The sudden change in size between September and October or November is due to maturation of young but large males. In September collections, males with partly formed gonopodia are often larger than the largest mature males, and it is likely that these fish are representative of large males obtained during the following months. Mature males make up 5-9% of the measurable sample (= mature males and all fish over 25 mm S. L.) from July to September, but 13 and 19% in October.

Small males are abundant in July, August, and September. The size distribution changes abruptly between June and July at about the same time as young-of-the-year females begin to have ova. Although it is not possible to use size to determine whether a specific male is a winter or a summer male, the changes are rather abrupt, with few large winter males in the August collections and few small summer males in the November collections.

The size distribution data for heterochir do not show equivalent changes in modal frequencies (fig. 31). The major size difference is a diminution in modal size at the beginning of the sample interval. Males may have been subjected to little predation above the dam, and the frequency of large males might reflect survival of the largest males over a prolonged interval. Smaller males would have been
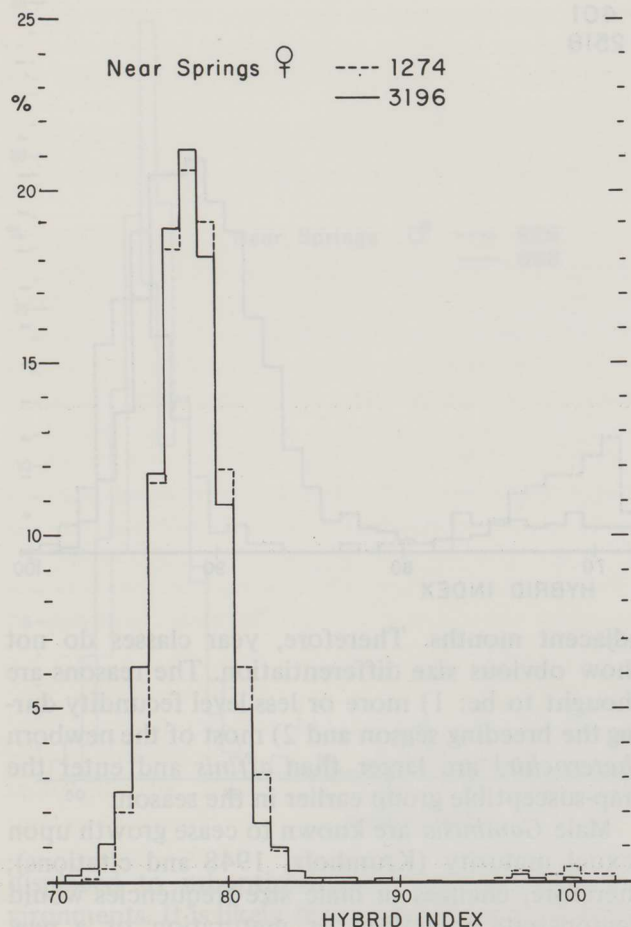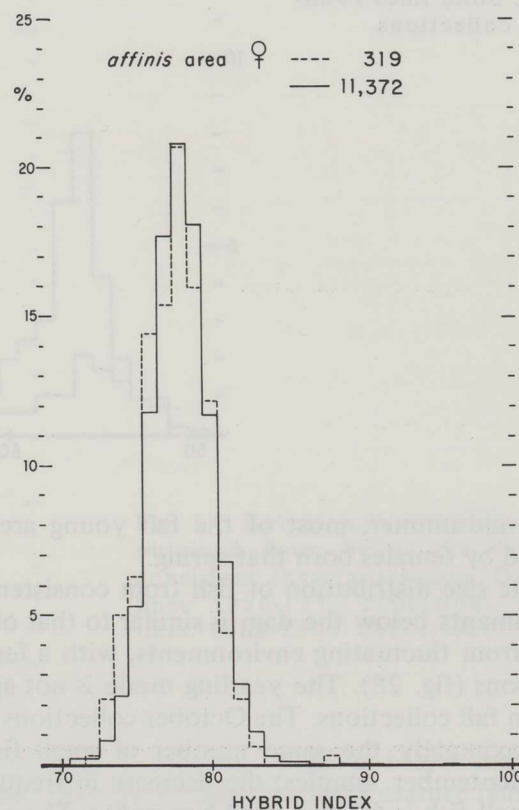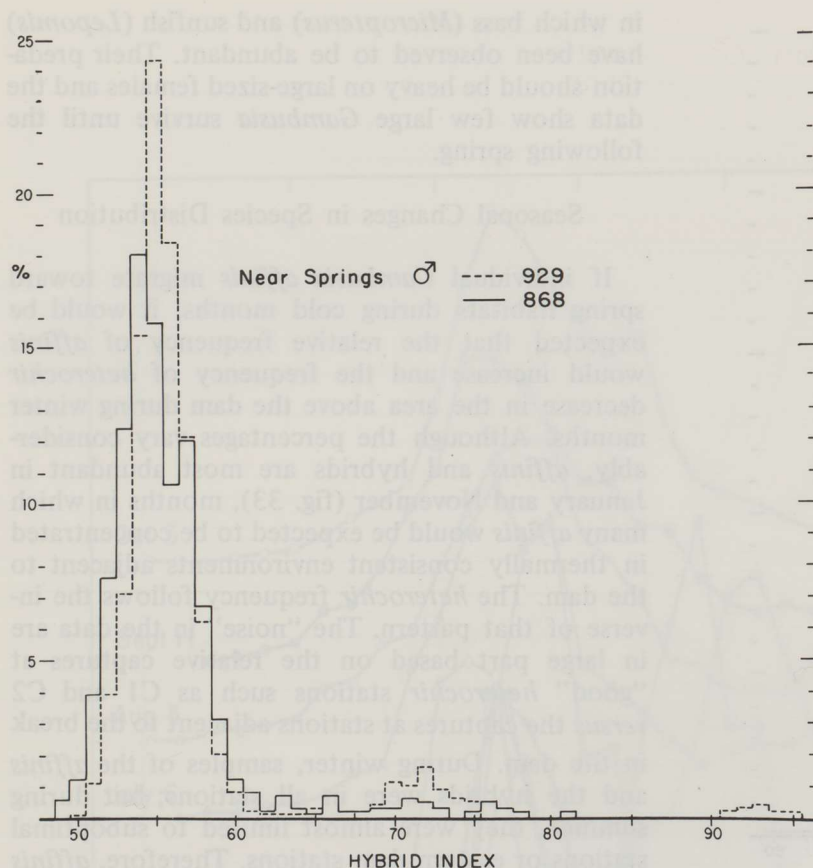
Fig. 24. Hybrid indices for all males obtained from below the dam in consistent thermal stations. Dashed lines 1956-1957 collections. Solid lines 1968-1969 collections.

displaced to suboptimal and predation-prone environments. It is likely that a winter group of males is present above the dam because many immature males were obtained in September but few immature males were collected in October, and the percentages of measured fish that were males were 13 and 22 in September and 47 and 48 in October. Again the male frequency was low all summer (11-26%) and high in winter (59-90%). The increase in total number of males in June may reflect a group of summer males. The change from 11% males in May to 19% in June supports this hypothesis.

It would be strategic for a fish to follow the male size changes shown for *G. affinis*. In winter little reproduction (and courtship?) is in progress. A maturing male that delayed masculinization would attain a large size and peck-order dominance. There would be no selective advantage in maturing quickly and entering the potential breeding population when reproduction was at a minimum. In contrast, during midsummer numerous young females are entering the breeding population and the few surviving winter males would not be likely to be able to service each young female. A genetic male that masculinized early could have many available females and leave more progeny than one that masculinized later and did not inseminate females in midsummer.

Seasonal Distribution of Fish

The change in frequency of large fish at steno-

thermal and eurythermal stations below the dam suggests that many fish (and most mature females) move to stenothermal environments during cold months. The relative number of fish obtained at these stations supports a migration hypothesis (fig. 32). Between January and May more than half of the fish were at stenothermal stations; between June and November more than two-thirds were at eurythermal stations. The December sample is intermediate. The small size of the May sample makes the precision of that part of the data dubious which permits a hypothesis that the fish were returning to the eurythermal stations in May. The migrations seem to involve primarily large fish because only winter samples have the millimeter percentage greater than the individual percentage at stenothermal stations.

Because temperature affects activity, it is possible to hypothesize that the absence of fish in traps at eurythermal stations in winter results from cold-induced quiescence. This is unlikely for two reasons: 1) The eurythermal stations were examined visually and virtually no *Gambusia* were observed, and 2) the stenothermal stations had their greatest numbers of fish in January when those stations recorded their lowest temperatures.

Gambusia, especially adults, seem to prefer warmer stations and migrate to stenothermal areas during cold winter months. The limited stenothermal environment would expose the *Gambusia* there to predation. Many adults would be expected to occupy suboptimal offshore areas (most of the fish taken in E2 through E7 were winter captures)
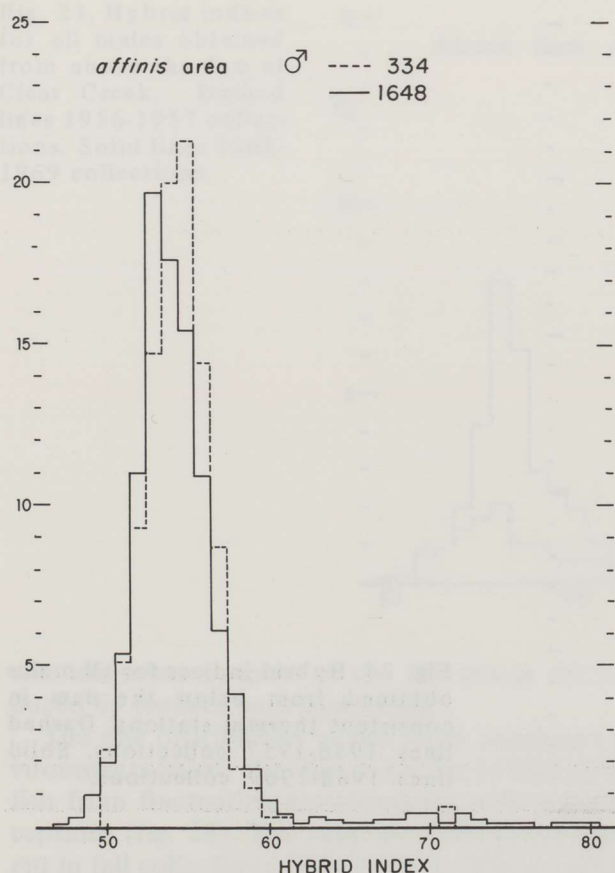
Fig. 25. Hybrid indices for all males obtained from below the dam in thermally fluctuating stations. Dashed lines 1956-1957 collections. Solid lines 1968-1969 collections.

in which bass (*Micropterus*) and sunfish (*Lepomis*) have been observed to be abundant. Their predation should be heavy on large-sized females and the data show few large *Gambusia* survive until the following spring.

Seasonal Changes in Species Distribution

If individual *Gambusia affinis* migrate toward spring habitats during cold months, it would be expected that the relative frequency of *affinis* would increase and the frequency of *heterochir* decrease in the area above the dam during winter months. Although the percentages vary considerably, *affinis* and hybrids are most abundant in January and November (fig. 33), months in which many *affinis* would be expected to be concentrated in thermally consistent environments adjacent to the dam. The *heterochir* frequency follows the inverse of that pattern. The "noise" in the data are in large part based on the relative captures at "good" *heterochir* stations such as C1 and C2 *versus* the captures at stations adjacent to the break in the dam. During winter, samples of the *affinis* and the hybrids were in all stations; but during summer, they were almost limited to suboptimal stations or eastern dam stations. Therefore, *affinis* not only move to consistent thermal habitats adjacent to the dam but also many pass through the break in the dam into typical *heterochir* environments.

The frequency of *heterochir* and hybrids below the dam is low at all times. Too few occurred at
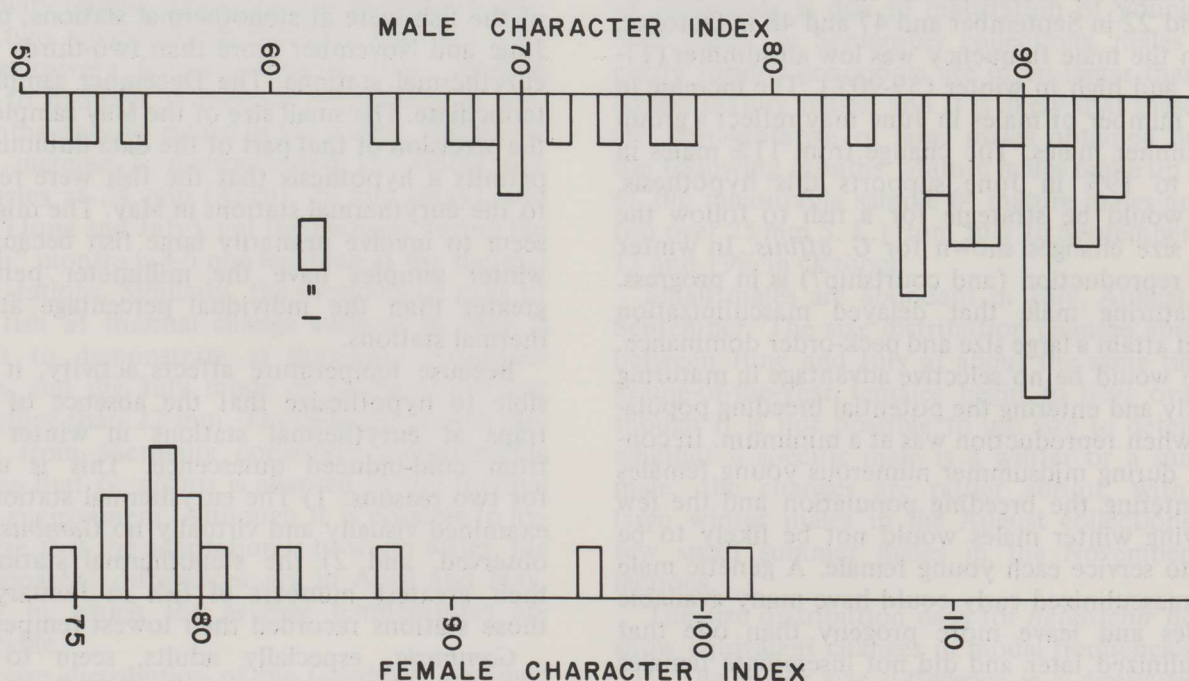
Fig. 26. Plots for index codes of males (above) and females (below) from sparsely populated areas above the dam at Clear Creek.

22
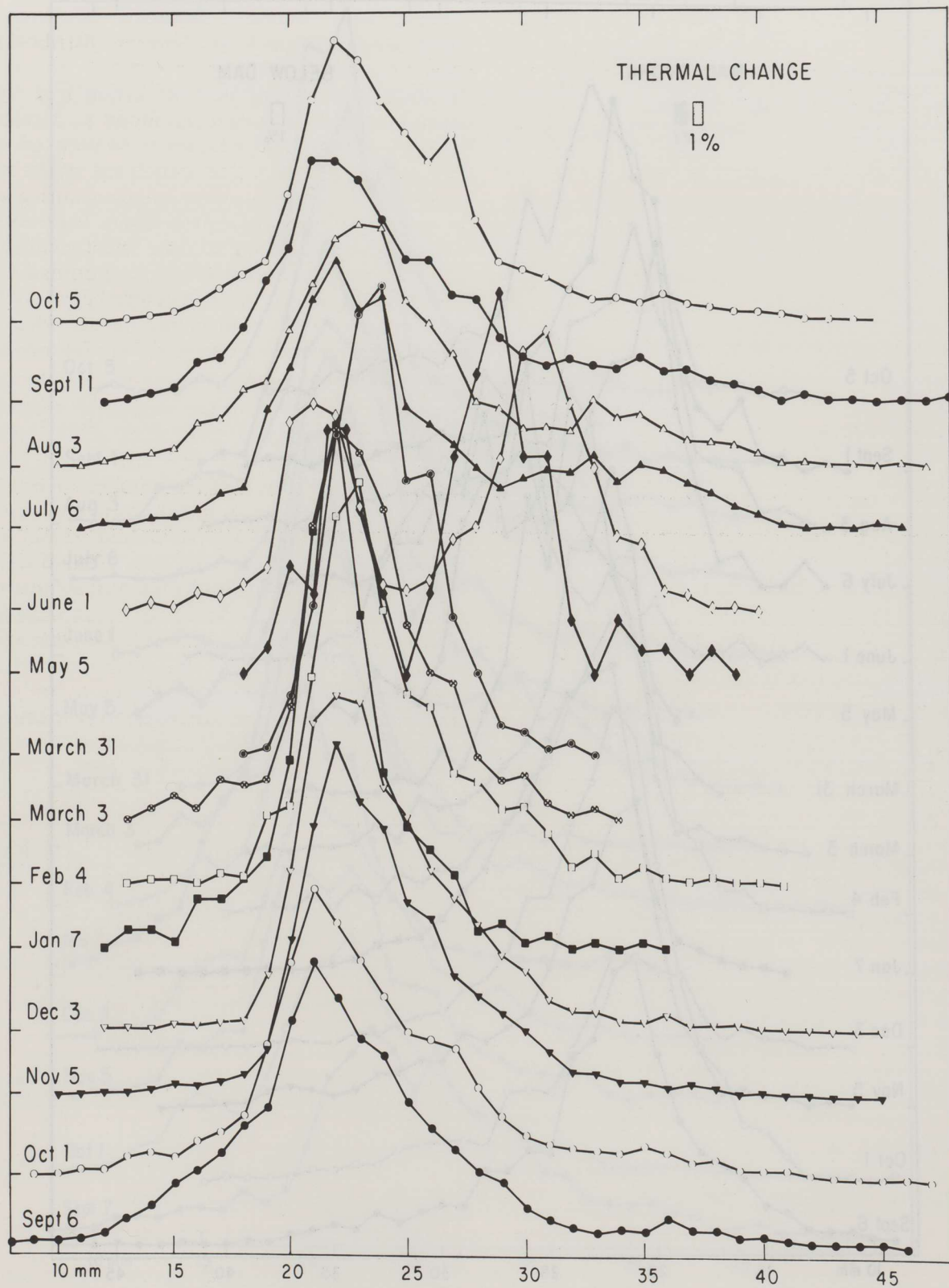
Fig. 27. Size distribution of *Gambusia* collected from thermally fluctuating environments below the dam.

23
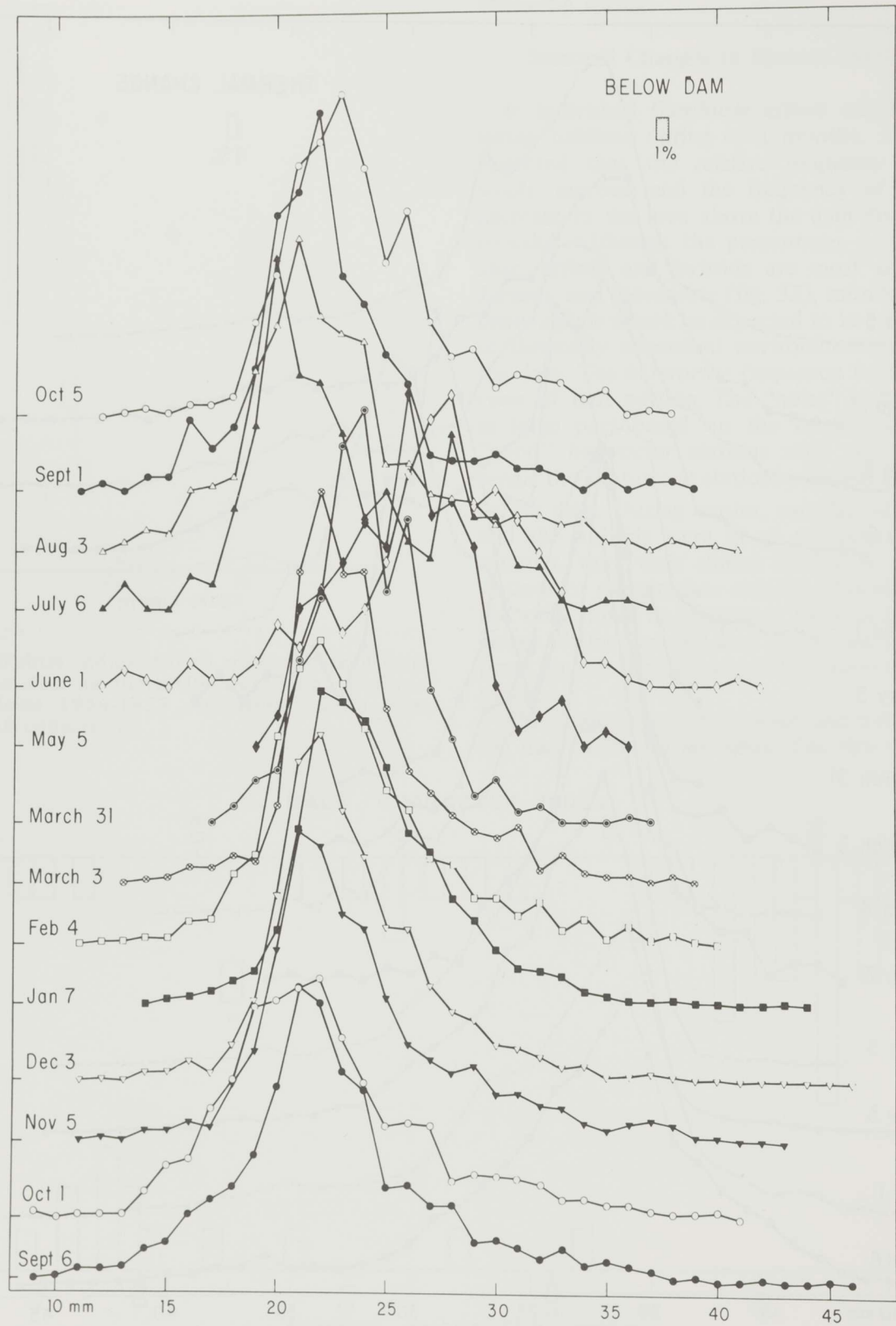
Fig. 28. Size distribution of *Gambusia* collected from thermally consistent environments below the dam.
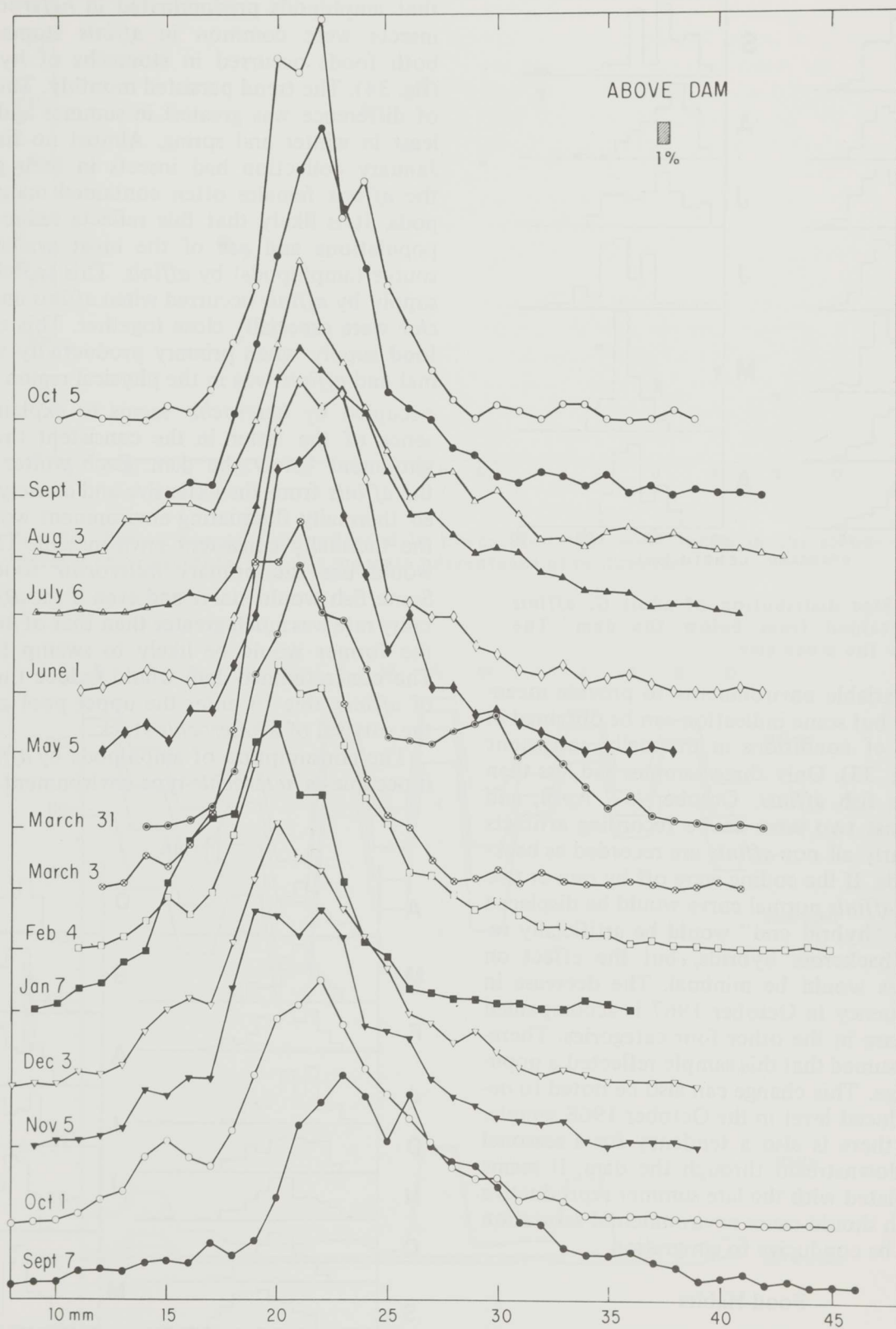
Fig. 29. Size distribution of *Gambusia* collected from above the dam.
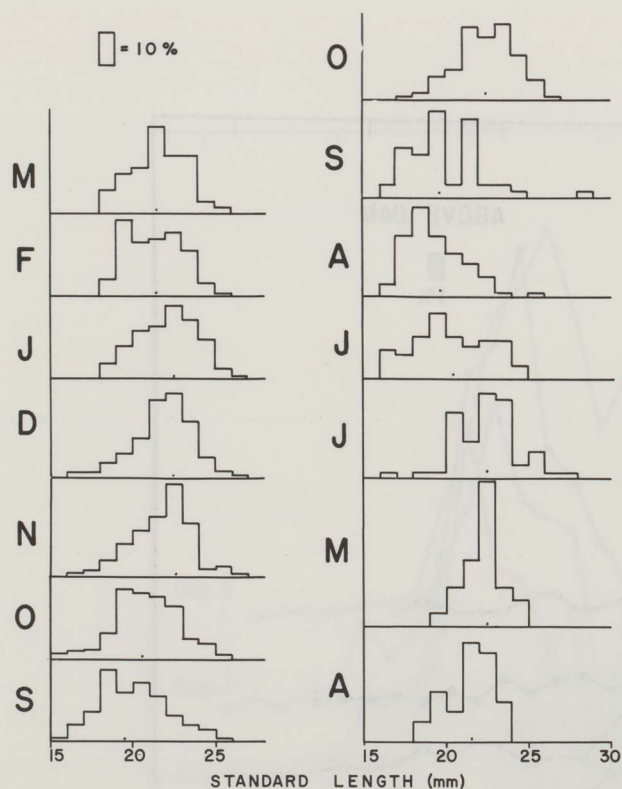
Fig. 30. Size distribution of adult *G. affinis* males obtained from below the dam. The dots show the mean size.

thermally variable environments to provide meaningful data, but some indication can be obtained in an analysis of conditions in thermally consistent stations (fig. 33). Only three samples had less than 90% of the fish *affinis:* October '67, April, and July. The last two seem to be recording artifacts because nearly all non-*affinis* are recorded as backcross hybrids. If the coding were off by one or two points, the *affinis* normal curve would be displaced so that the "hybrid end" would be artificially recorded as backcross hybrids, but the effect on other classes would be minimal. The decrease in *affinis* frequency in October 1967 is accompanied by an increase in the other four categories. Therefore, it is assumed that this sample reflected a population change. This change can also be noted to occur at a reduced level in the October 1968 sample. Therefore, there is also a tendency for a seasonal migration downstream through the dam. It seems to be associated with the late summer reproductive peaks which should cause environmental saturation that would be conducive to emigration.

Food Habits

The females over 25 mm (those analyzed for amount of hybridization) were also examined for food content. Because the fish were captured with baited traps that had been in the water six hours before the fish were preserved, most stomachs were filled with the bait (dog food). Many others con-

tained algae and/or detritus, which may have been ingested accidentally. Two other categories, amphipods and various insects, were present in large numbers of the guts. It soon became obvious that amphipods predominated in *heterochir* guts, insects were common in *affinis* stomachs, and both foods occurred in stomachs of hybrid fish (fig. 34). The trend persisted monthly. The amount of difference was greatest in summer and fall and least in winter and spring. Almost no fish in the January collection had insects in their guts, and the *affinis* females often contained many amphipods. It is likely that this reflects reduced insect populations and use of the most available food source (amphipods) by *affinis*. This switch in food supply by *affinis* occurred when *affinis* and *heterochir* were especially close together. This overlap in food supply when primary productivity was minimal and *affinis* was in the physical region typically occupied by *heterochir* seems to explain the absence of the latter in the consistent thermal environment below the dam. Each winter most of the *affinis* from the extensive and densely populated thermally fluctuating environment would enter the thermally consistent environment. There they would use the primary *heterochir* food source. Some fish would starve and even if the *affinis* mortality rate was much greater than that of *heterochir*, the former would be likely to swamp the latter. The concrete-lined dam would reduce the number of *affinis* able to enter the upper pool and insure the survival of a *heterochir* stock.

The consumption of amphipods by *affinis* when it occupies a *heterochir*-type environment in winter
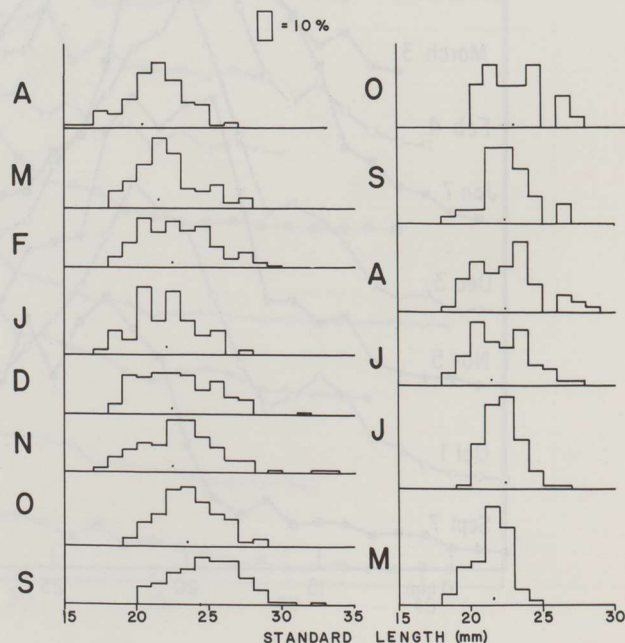
Fig. 31. Size distribution of adult *G. heterochir* males obtained from above the dam. The dots show mean size.
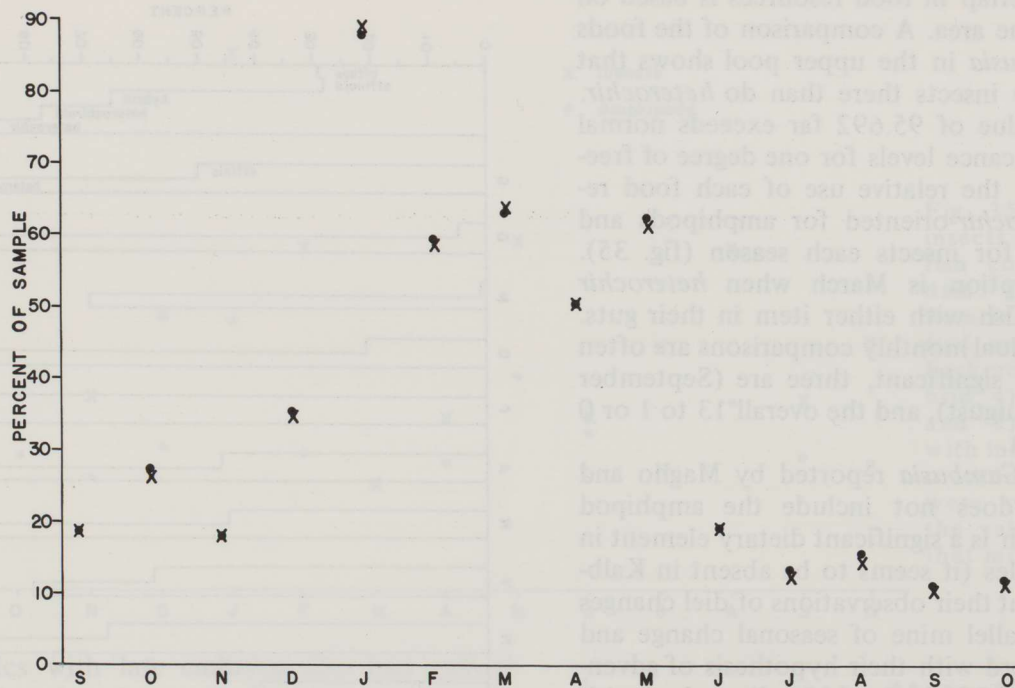
26

Fig. 32. Percentage of fish collected in lower pool that were taken in the steno-thermal environments. The X's indicate percentages of millimeters.
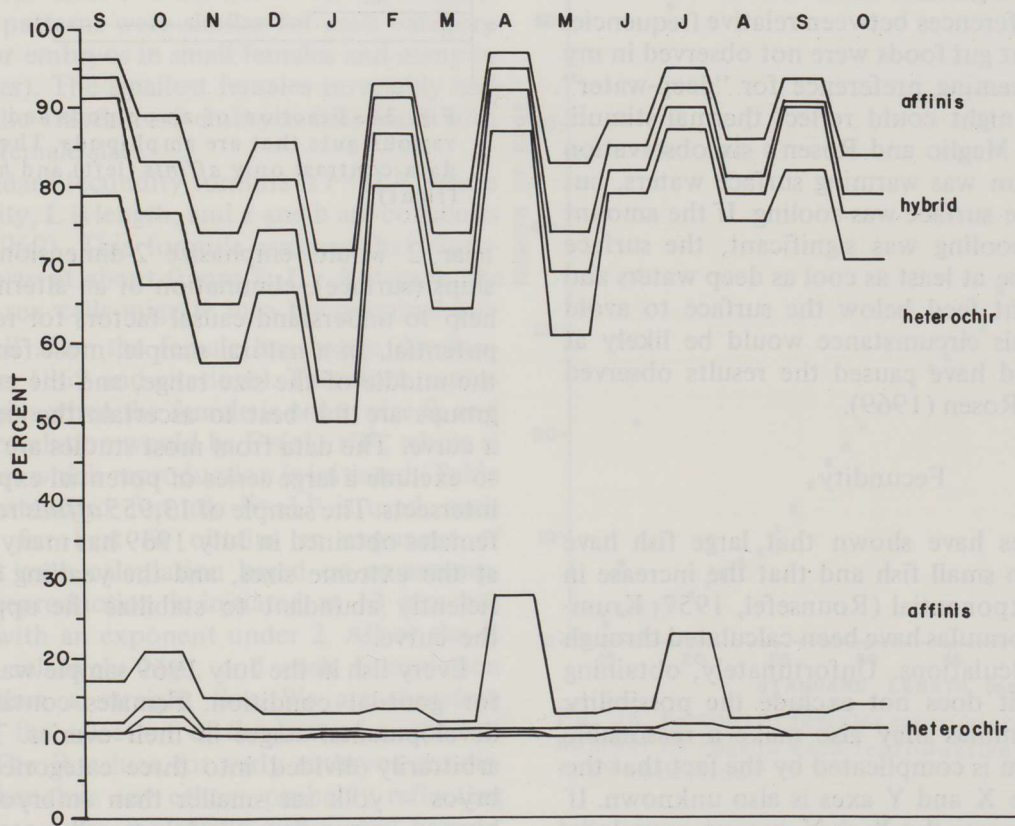

Fig. 33. Percentages of *affinis*, hybrids, *heterochir*, and intermediates in the above-dam collections (above) and thermally consistent environments below dam (below).

suggests the overlap in food resources is based on availability in the area. A comparison of the foods eaten by *Gambusia* in the upper pool shows that *affinis* eat more insects there than do *heterochir*. A chi-square value of 95.692 far exceeds normal statistical significance levels for one degree of freedom. Similarly, the relative use of each food resource is *heterochir*-oriented for amphipods and *affinis*-oriented for insects each season (fig. 35). The only exception is March when *heterochir* were the only fish with either item in their guts. Although individual monthly comparisons are often not statistically significant, three are (September '67, July, and August), and the overall 13 to 1 or 0 is also significant.

The diet of *Gambusia* reported by Maglio and Rosen (1969) does not include the amphipod *Gammarus* which is a significant dietary element in all of our samples (it seems to be absent in Kalbfleisch Pond), but their observations of diel changes in ingestion parallel mine of seasonal change and both are in accord with their hypothesis of adventitious feeding. The night foods are discordant with my conclusion of night inactivity; however, if their night sample was collected at 2200 hours (their observation period), the foods in the guts might reflect feeding near dusk (later in clock hours at northern latitudes). I assumed that food in the gut could reflect ingestion as much as four hours earlier. The differences between relative frequencies in day and night gut foods were not observed in my studies. The seeming preference for "deep-water" food items at night could reflect thermal stimuli. During five of Maglio and Rosen's six observation intervals the sun was warming surface waters, but during dusk the surface was cooling. If the amount of nighttime cooling was significant, the surface waters would be at least as cool as deep waters and *Gambusia* might feed below the surface to avoid cold water. This circumstance would be likely at dusk and could have caused the results observed by Maglio and Rosen (1969).

Fecundity

Many studies have shown that large fish have more eggs than small fish and that the increase in fecundity is exponential (Rounsefel, 1957; Krumholz, 1948). Formulas have been calculated through least-square calculations. Unfortunately, obtaining a reasonable fit does not exclude the possibility that other formulas may also make a reasonable fit. The problem is complicated by the fact that the 0 point on the X and Y axes is also unknown. If the curve intersects the X or Y axes at one place, the exponent of fecundity increase could be 3, but if the size for 0 eggs is larger, an exponent of 2 would produce a better fit than 3 (Hubbs, *et al.*, 1968). Because exponents near 3 indicate three dimensions (volume) are critical and exponents
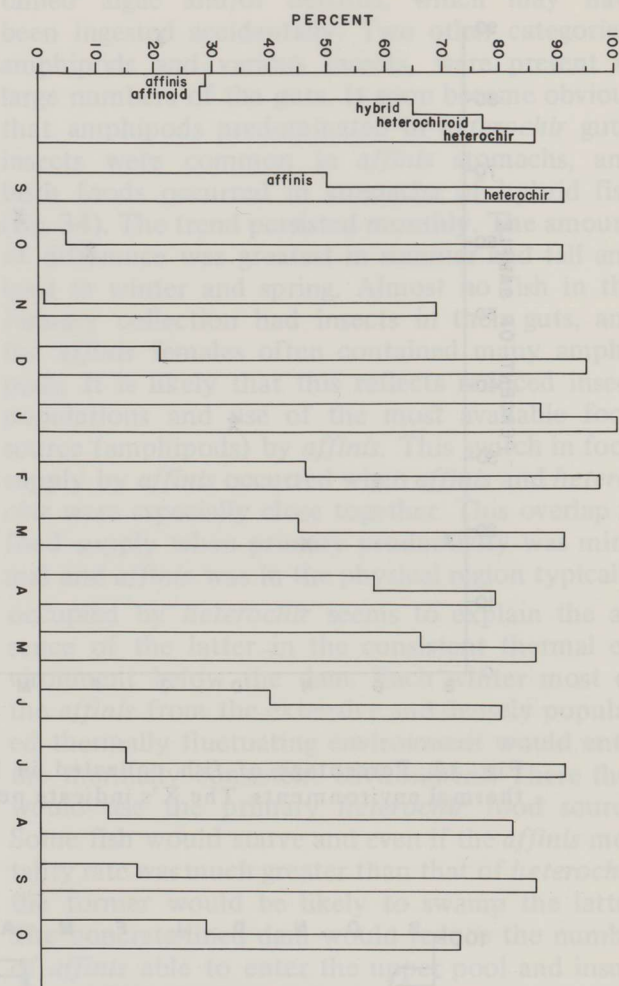
Fig. 34. Fraction of amphipods and insects in various guts that are amphipods. The monthly data contrast only *affinis* (left) and *heterochir* (right).

near 2 would emphasize 2-dimensional relationships (surface), elimination of an alternative might help to understand causal factors for reproductive potential. In a natural sample, most females are in the middle of the size range, and the extreme size groups are the best to ascertain the exponent for a curve. The data from most studies are inadequate to exclude a large series of potential exponents and intersects. The sample of 13,955 *affinis* reproductive females obtained in July 1969 has many individuals at the extreme sizes, and the yearling class is sufficiently abundant to stabilize the upper end of the curve.

Every fish in the July 1969 sample was examined for gonadal condition. Females contained many developmental stages in their ovaries. These were arbitrarily divided into three categories: late embryos — yolk sac smaller than embryo; early embryos — eyes pigmented but yolk sac larger than embryo; and eggs — egg diameter greater than 1 mm but eyes not pigmented. Krumholz (1948, 1963) suggested that the last category did not occur in the *Gambusia* he studied; many, however, were found among our specimens. Very frequently
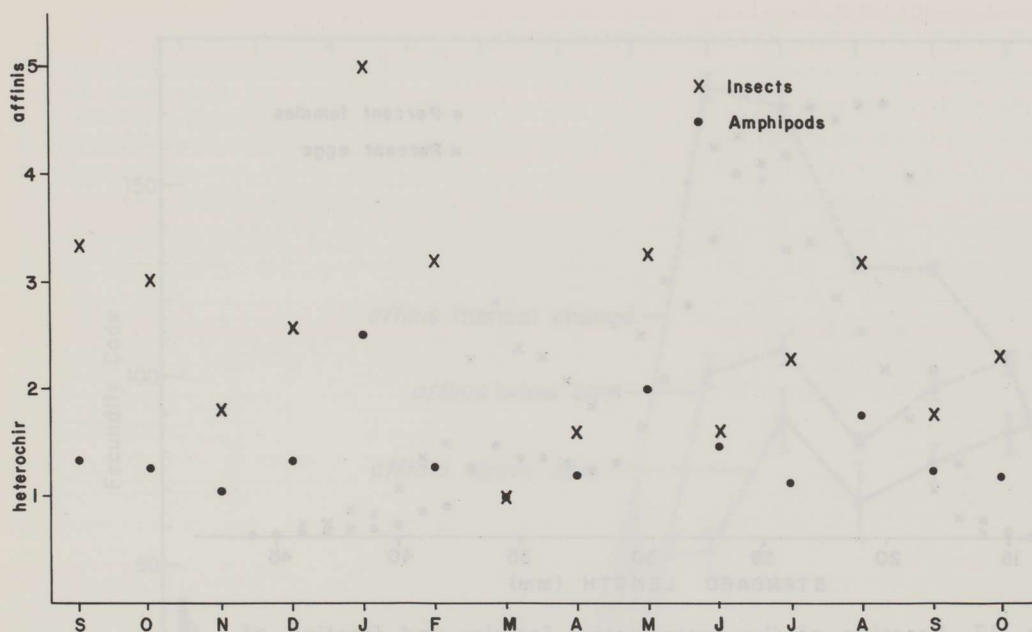
Fig. 35. Relative use of insects and amphipods by fish collected above the dam. Each fish with an item in its gut was classified as 1) *heterochir,* 2) backcross hybrid, 3) hybrid, 4) backcross hybrid, and 5) *affinis.* All fish with insects or amphipods recorded in that month were averaged to obtain the values for that item that month.

the females with late embryos also had yolked eggs smaller than 1 mm in diameter and, on occasion, larger. Unless the late embryos were drastically less numerous than the eggs, the number of late embryos was used in subsequent calculations. This reduced potential problems of eggs resorption (Vladykov, 1956) and problems of low counts resulting from birth of a fraction of an egg complement. The patterns were similar for each category (few eggs or embryos in small females and many in large females). The smallest females invariably had eggs, and the fraction of embryos increased with increase in female size.

The standard fecundity formula is $F=aL^b$, where F is fecundity, L is length, and a and b are constants (Blaxter, 1969). This formula assumes that reproduction starts at about 0 mm S. L.; however, the primordial sex cells migrate into the germinal epithelium well after the female has begun development (Hoar, 1969 and citations). Therefore, reproduction begins when the female is not at size 0, and a better formulation would be $F=(aL-ri)^b$, where ri is the size at which reproduction is initiated (Table 5). If the standard formula $F=aL^b$ is used, each calculation for best fit obtains an exponent of over 3; yet each calculation based on an assumption that reproduction is initiated at 13 mm has a best fit with an exponent under 2. All of the R values are relatively high, and each formulation is better than a straight line. We are therefore faced with the selection of the best of a series of best fits. The R values for early embryos are distinctly higher than any others, probably reflecting less influence from resorption and partial broods. Among the early embryo data, ri = 11 has the highest R value, and therefore qualifies as the best fit of the series. Its exponent is 1.90 — close to the value of 2 expected for surface-related control. A value slightly below 2 would be expected if in-
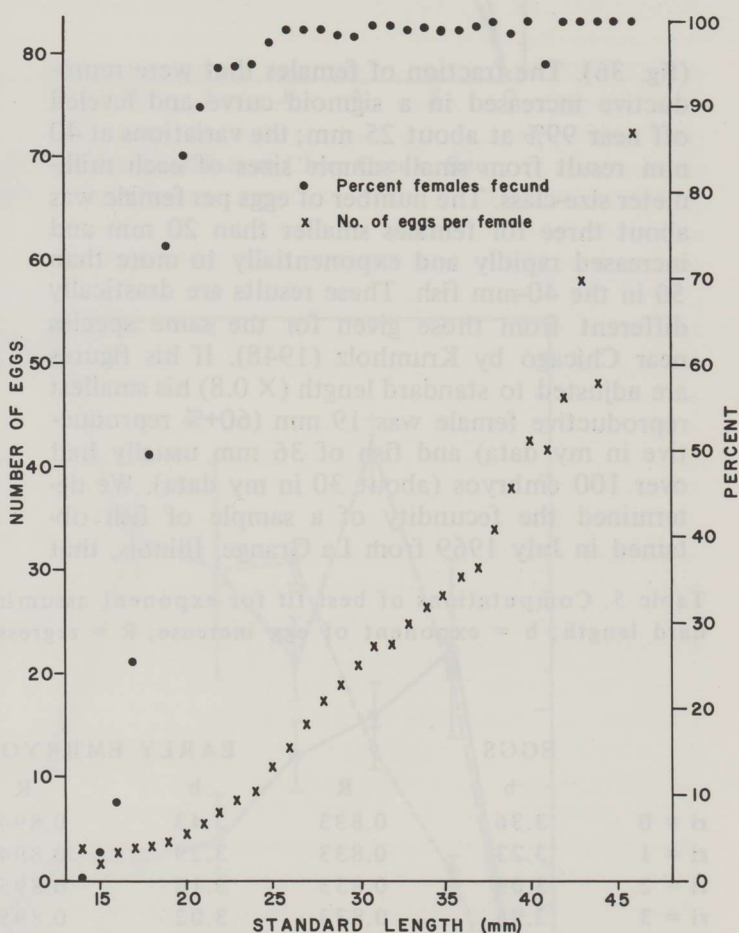
Fig. 36. Percent of females reproductive and average number of eggs in each reproductive female in July 1969 collection.

take was squared and maintenance needs cubed with increase in female size. Therefore, a two-dimensional element (= food absorption) is a critical limitation to fecundity.

None of the fish less than 14 mm had mature ova, and few were mature in the 16 mm size class

29
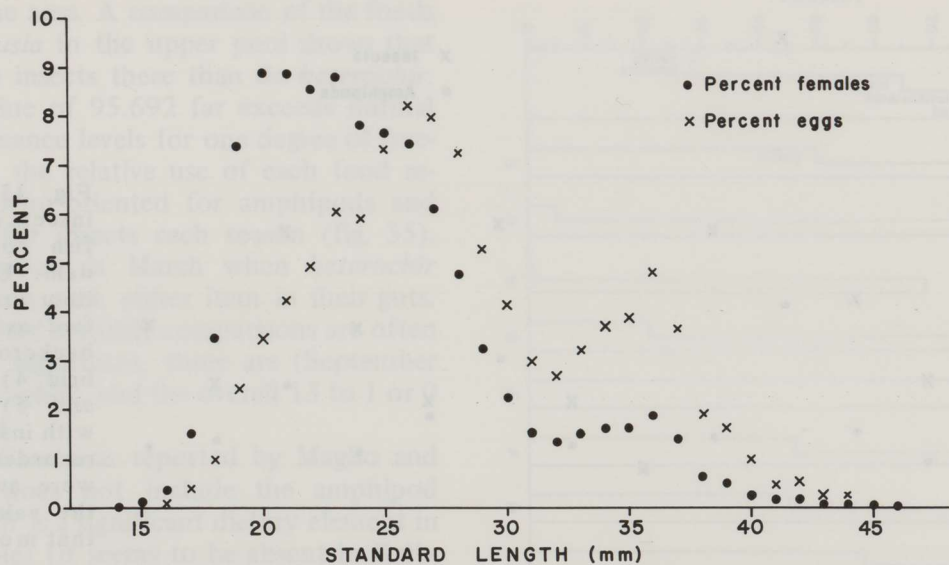
Fig. 37. Fraction of the reproductive females and fraction of young by female size class in July 1969 collection.

(fig. 36). The fraction of females that were reproductive increased in a sigmoid curve and leveled off near 99% at about 25 mm; the variations at 40 mm result from small sample sizes of each millimeter size-class. The number of eggs per female was about three for females smaller than 20 mm and increased rapidly and exponentially to more than 50 in the 40-mm fish. These results are drastically different from those given for the same species near Chicago by Krumholz (1948). If his figures are adjusted to standard length (× 0.8) his smallest reproductive female was 19 mm (60+% reproductive in my data) and fish of 36 mm usually had over 100 embryos (about 30 in my data). We determined the fecundity of a sample of fish obtained in July 1969 from La Grange, Illinois, that

descended from the Argonne Woods stock studied by Krumholz and found brood sizes in excess of 100 in all fish of 40 mm S. L. showing that the difference is between the samples, not due to technique. Although I would have preferred to use fish from the environments studied by Krumholz to avoid environmental effects, urbanization prevented my attaining this objective. Nevertheless, Chicago-area fish are much larger and more fecund at any given size than are those from Clear Creek. My data are similar to Krumholz's data on *G. manni* from Bimini (1963) in which females may mature at 16 mm, but *G. manni* have even fewer young per brood (10 or fewer for 28-mm females).

The 13,955 females are not evenly distributed with size. Most are in the 19-26-mm size classes

Table 5. Computations of best fit for exponent assuming reproduction begins at various sizes in mm standard length; b = exponent of egg increase; R = regression correlation; ri = size at reproductive initiation.

	EGGS		EARLY EMBRYOS		LATE EMBRYOS		TOTAL	
	b	R	b	R	b	R	b	R
ri = 0	3.36	0.833	3.43	0.894	3.47	0.878	3.43	0.865
ri = 1	3.23	0.833	3.29	0.894	3.33	0.879	3.29	0.866
ri = 2	3.09	0.833	3.16	0.895	3.19	0.879	3.16	0.866
ri = 3	2.95	0.833	3.02	0.895	3.05	0.879	3.02	0.866
ri = 4	2.81	0.833	3.89	0.896	2.92	0.880	2.88	0.866
ri = 5	2.67	0.833	2.75	0.897	2.78	0.880	2.74	0.867
ri = 6	2.53	0.833	2.61	0.897	2.64	0.880	2.60	0.867
ri = 7	2.39	0.833	2.48	0.898	2.50	0.881	2.46	0.867
ri = 8	2.24	0.833	2.34	0.899	2.35	0.881	2.31	0.867
ri = 9	2.09	0.832	2.19	0.899	2.21	0.881	2.16	0.867
ri = 10	1.94	0.831	2.04	0.899	2.06	0.881	2.02	0.867
ri = 11	1.79	0.830	1.90	0.900	1.91	0.880	1.87	0.866
ri = 12	1.63	0.827	1.75	0.899	1.76	0.880	1.71	0.865
ri = 13	1.46	0.823	1.59	0.898	1.60	0.878	1.54	0.862

30

Fig. 38. Relative seasonal fecundity of *Gambusia affinis* at Clear Creek. See text for explanation of fecundity code.
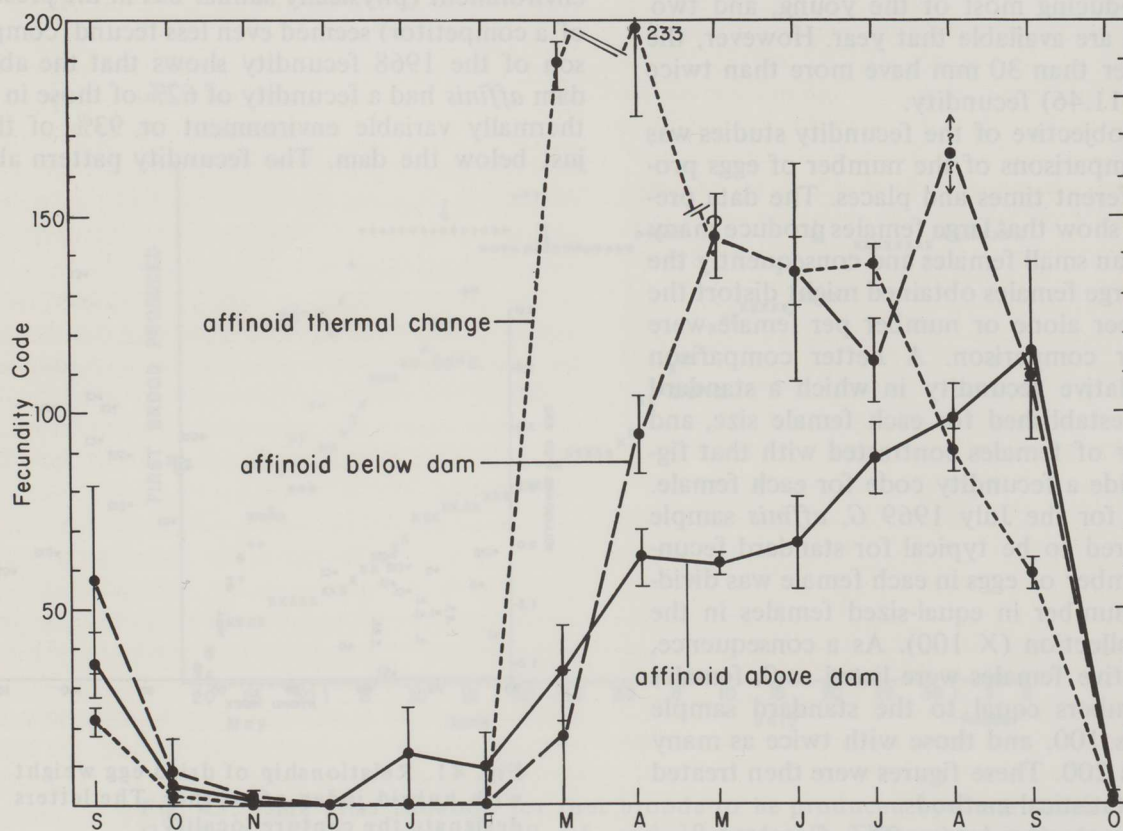
Fig. 39. Relative seasonal fecundity of hybrids similar to *Gambusia affinis* at Clear Creek. See text for explanation of fecundity code.

31
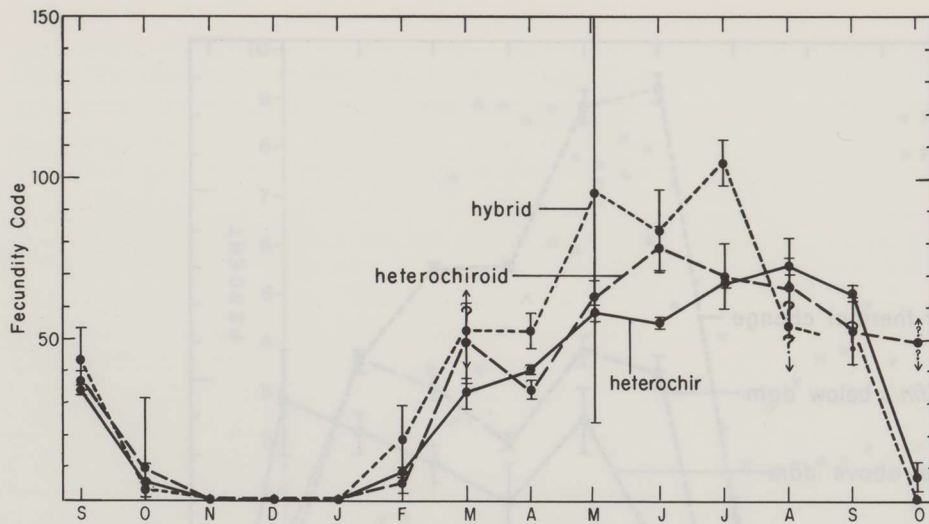
Fig. 40. Relative seasonal fecundity of *Gambusia heterochir* and hybrids collected from the upper pool at Clear Creek. See text for explanation of fecundity code.
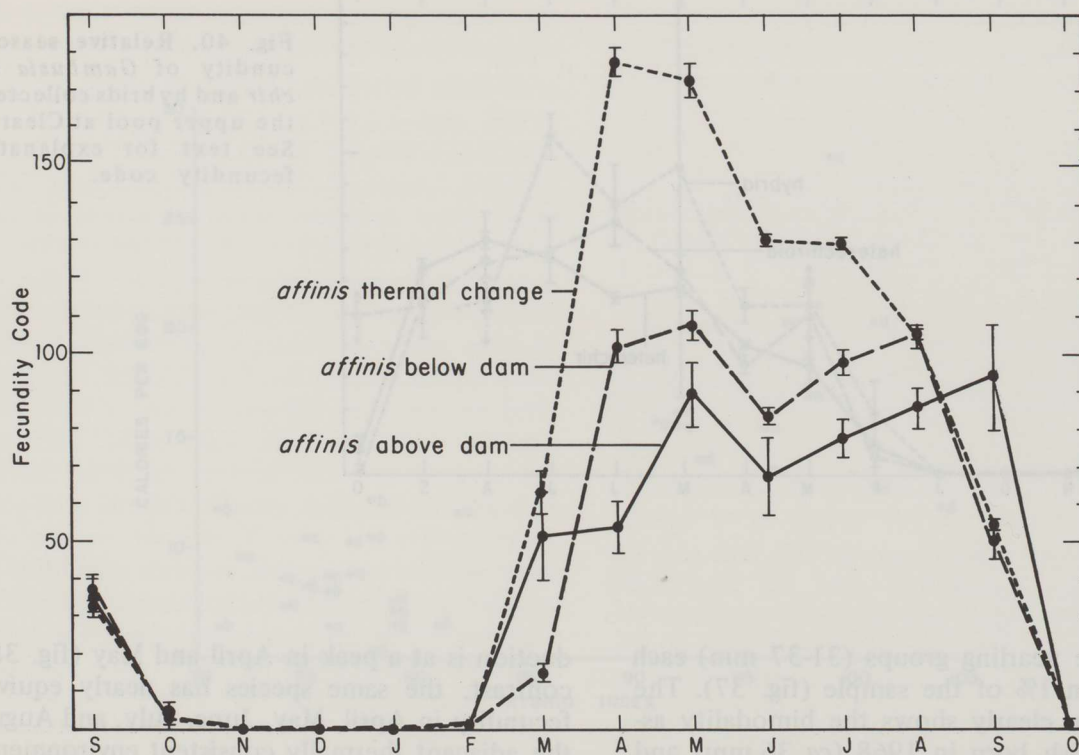
(6-9%), but the yearling groups (31-37 mm) each have more than 1% of the sample (fig. 37). The size distribution clearly shows the bimodality associated with fish born in 1968 (*ca* 36 mm) and 1969 (*ca* 20 mm). The bimodality is even more pronounced if the fractions of the 159,986 embryos are plotted. The increased fecundity of the larger size groups makes that peak pronounced.

The plot also shows that by August young-of-the-year are producing most of the young, and two more broods are available that year. However, the females longer than 30 mm have more than twice the average (11.46) fecundity.

The basic objective of the fecundity studies was to obtain comparisons of the number of eggs produced at different times and places. The data presented above show that large females produce many more eggs than small females and consequently the number of large females obtained might distort the data if number alone or number per female were the basis for comparison. A better comparison would be relative fecundity in which a standard fecundity is established for each female size, and the fecundity of females contrasted with that figure will provide a fecundity code for each female.

The plots for the July 1969 *G. affinis* sample were considered to be typical for standard fecundity. The number of eggs in each female was divided into the number in equal-sized females in the July 1969 collection (X 100). As a consequence, non-reproductive females were listed as 0, females with egg numbers equal to the standard sample were listed as 100, and those with twice as many were listed as 200. These figures were then treated by standard statistical methods.

At Clear Creek, *Gambusia affinis* females are reproductive from March through September, and reproduction is nil in November, December, and January. In the thermal change environment, repro-

duction is at a peak in April and May (fig. 38). In contrast, the same species has nearly equivalent fecundity in April, May, June, July, and August in the adjacent thermally consistent environment below the dam. In addition, in 1968 the overall fecundity of thermally consistent females was 67% of that of those from thermally fluctuating nearby localities (as measured by the area above the base line). The *G. affinis* females from the *G. heterochir* environment (physically similar but in the presence of a competitor) seemed even less fecund; comparison of the 1968 fecundity shows that the above-dam *affinis* had a fecundity of 62% of those in the thermally variable environment or 93% of those just below the dam. The fecundity pattern above
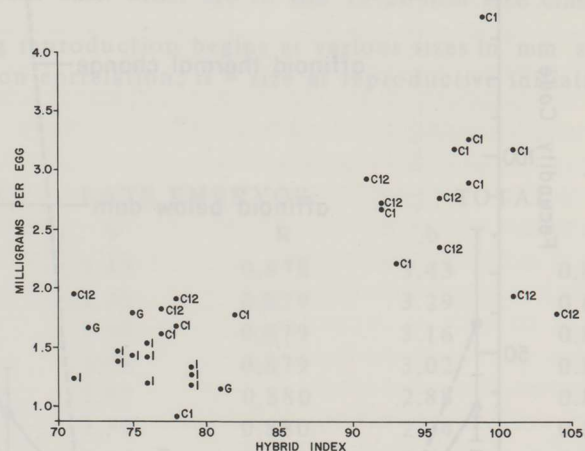
Fig. 41. Relationship of dried egg weight with hybrid index of mother. The letters designate the capture locality.

32
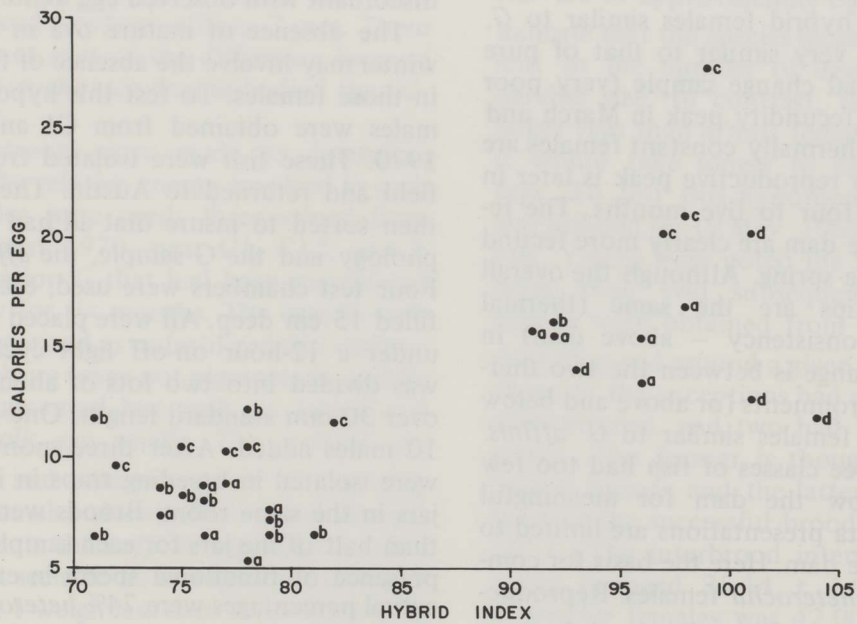
Fig. 42. Relationship of calories in average egg with hybrid index of mother; a = late embryos, b = middle embryos, c = early embryos, d = unfertilized eggs. The methodology for obtaining caloric data follows Phillipson (1964).
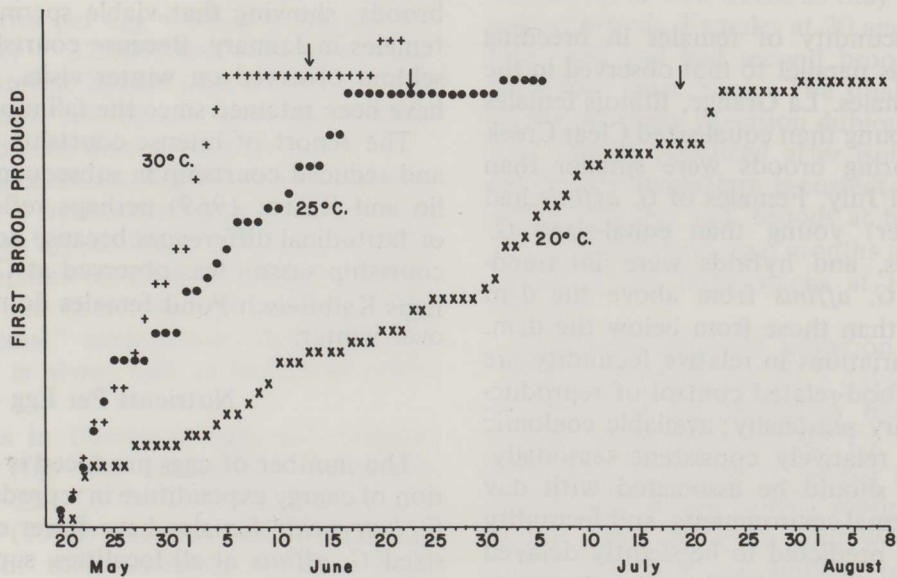
Fig. 43. Time after isolation for first broods to be produced by Clear Creek *Gambusia affinis* females at 20, 25, and 30-degree C. The arrows indicate average interbrood intervals. The X axis shows the relative frequency of broods produced. Because few were available at 30-degree C the steps are larger than those at 20 and 25-degree C.

33

the dam shows a trend toward a fall peak. All samples begin and end reproduction at about the same date, suggesting light rather than temperature as a primary control of reproductive season.

The fecundity of hybrid females similar to *G. affinis* has a pattern very similar to that of pure *G. affinis*. The thermal change sample (very poor sample) has an early fecundity peak in March and April (fig. 39). The thermally constant females are less fecund and their reproductive peak is later in the year and covers four to five months. The females from above the dam are clearly more fecund in the fall than in the spring. Although the overall fecundity relationships are the same (thermal change − thermal consistency − above dam) in sequence, the big change is between the two thermally consistent environments (or above and below the dam) in hybrid females similar to *G. affinis*.

The remaining three classes of fish had too few examples from below the dam for meaningful discussion, so the data presentations are limited to those from above the dam. Here the basis for comparison should be *G. heterochir* females. Reproduction is nil in November, December, and January and gradually increases in February, March, April, and May. Fecundity is nearly equal or increases slightly until August and then declines in September and October (fig. 40). The pattern for hybrids similar to *heterochir* and for intermediate hybrids is similar. Although the variations may be by chance, it seems that fecundity is higher if the fish are phenotypically more similar to *affinis*; however, the *affinis* fecundity equals that of hybrids. If we assume the 1968 fecundity of *heterochir* equals 100, the other four classes are 119, 131, 139, and 131 respectively.

The relative fecundity of females in breeding traps in Austin was parallel to that observed in the field-preserved females. La Grange, Illinois females produced more young than equal-sized Clear Creek females. Early spring broods were smaller than those in June and July. Females of *G. affinis* had more (but smaller) young than equal-sized *G. heterochir* females, and hybrids were intermediate. Females of *G. affinis* from above the dam had fewer young than those from below the dam.

The seasonal variations in relative fecundity are in accord with a food-related control of reproduction. Food can vary seasonally; available coelomic space should be relatively consistent seasonally. Food abundance should be associated with day length in stenothermal environments, and fecundity changes would be predicted to be slightly delayed because of problems associated with processing metabolites. In eurythermal environments, the environmental metabolism would be low in midwinter and essential minerals could be "stockpiled", and used up in an early reproductive peak. Both hypothetical patterns fit the observed fecundity in that environment. In contrast, any changes in available coelomic space would result from food in the gut or associated fat deposits. Both should occupy space when fecundity is high and consequently are discordant with observed egg numbers.

The absence of mature ova in females in midwinter may involve the absence of functional sperm in those females. To test this hypothesis, adult females were obtained from C1 and G in January 1970. These fish were isolated from males in the field and returned to Austin. The C1 sample was then sorted to insure that all had *heterochir* morphology and the G sample, the *affinis* phenotype. Four test chambers were used, each 111 × 45 cm filled 15 cm deep. All were placed in a 25° C room under a 12-hour on-off light cycle. Each sample was divided into two lots of about 60 females all over 30 mm standard length. One of each pair had 10 males added. After three months, the females were isolated in breeding traps in individual gallon jars in the same room. Broods were found in more than half of the jars for each sample, indicating the presence of functional sperm in each sample. The actual percentages were 74% *heterochir* with males, 65% without males, 57% *affinis* with males, and 55% without males. In each comparison the brood frequency is slightly higher in females exposed to males, and four of the five females that produced non-fertilized eggs had been isolated from males, showing that the presence of males would help increase fertilization. On the other hand, the non-fertilized eggs were produced after four months isolation and could have happened in similar experiments begun in midsummer (reproductive peak). Despite the occasional production of non-fertilized eggs, most females produced normal broods, showing that viable sperm are present in females in January. Because courtship activity was seldom observed on winter visits, this sperm may have been retained since the fall months.

The report of intense courtship activity in May and reduced courtship in subsequent months (Maglio and Rosen, 1969) perhaps reflects population or latitudinal differences because no similar intense courtship season was observed at Clear Creek. Perhaps Kalbfleisch Pond females do not retain sperm over winter.

Nutrients Per Egg ·

The number of eggs produced is only one reflection of energy expenditure in reproduction. Because *G. heterochir* females have fewer eggs than equal-sized *G. affinis* at all localities, superficially it appears as if the latter always have more excess metabolites available for reproduction. In contrast, *G. heterochir* may invest enough energy in each egg to compensate for the number difference. Casual observations during egg counts indicated that each *heterochir* egg had more energy value than an *affinis* egg. For example, the average diameter of

mature *G. heterochir* eggs in the September 1967 preserved sample was 1.75 mm as compared to 1.42 mm for *G. affinis* eggs in the same sample. In addition, newborn young *heterochir* are 9 mm standard length and newborn *affinis,* 7 mm. These figures only reflect part of the difference because individual *G. heterochir* are deeper-bodied than *G. affinis* at all sizes.

Two measurements were made to determine more precisely the relative energy involved in each egg. Four samples were used: three seined from Clear Creek August 1970, near C1, C12, and I, and one from station G that had been maintained in the laboratory for six months. The eggs in each female were categorized as unfertilized (no embryo visible), early embryo (eyes not pigmented), middle embryo (eyes pigmented but yolk sac large), and late embryo (yolk sac small). The average egg weight (fig. 41) and caloric content of eggs (fig. 42) of the 33 females shows that *heterochir* eggs have more nutrients than *affinis* eggs. The locality sampled does not seem to be as important although *affinis* eggs from I weigh less than those from C12; however, C1 has both high and low figures. The developmental stage seems to have some impact on caloric content. Only four females with unfertilized eggs were examined and all had fewer calories than would otherwise be expected; the egg weights were also low. Because it is not possible to ascertain if the relationship of egg nutrient to hybrid index code is exponential, simple regressions were calculated both with and without the data from females with unfertilized eggs. The formulas are: number of calories per egg = 0.375 (maternal hybrid code index) – 19.631 with, and without, number of calories per egg = 0.530 (maternal hybrid code index) – 32.009; and micrograms per egg = 0.077 (maternal hybrid code index) – 4.509 with, and without, micrograms per egg = 0.083 (maternal hybrid code index) – 4.897.

Each of the calculations results in about a doubling of values at a *heterochir* index code (*ca* 100) compared with an *affinis* index code (*ca* 77). This calculation compares favorably with the relationship of *affinis* and *heterochir* fecundity in their respective "optimal" environment in which contrast *heterochir* is about half as fecund as *affinis* (actually 52%).

The nutrients in *Gambusia* eggs and embryos are equivalent (fig. 41) throughout incubation (35 + days at 25° C). In contrast, Paffenhöfer and Rosenthal (1968) showed that herring eggs lost one-third of their nutrients during a much shorter incubation period (18 days at 8° C). Because *Gambusia* ontogeny must use nutrients, and weights are equivalent throughout ontogeny, I agree with Scrimshaw (1945), who studied only dry weight, that nutrients are made available to the embryos while inside of their mothers.

The breeding seasons for *G. affinis* and *G. heterochir* are of approximately equal length. This would indicate that the species fecundity would be equivalent to the number of eggs in females of comparable size. In contrast, female *heterochir* have larger eggs than *affinis* females, and it is likely that it would take longer to produce the *heterochir* eggs. To test this hypothesis, a series of females was isolated on 19 May 1969 to study brood intervals. Young were noted the following day and for more than two subsequent months. Replicate broods were obtained from several females. Most were clumped around a given time between broods. Many of the exceptions had about double the standard interval, and two had broods very close together. The former is thought to represent overlooked broods and the latter, rare superfoetation. Most of the successful brood intervals were run at 25° C. The interbrood interval for the *affinis* females averaged 35.14 ± 1.978 and that of the *heterochir* females was 42.00 ± 2.142. The probability that the difference is not significant is less than 0.01.

Although 25° C is a common summer temperature near Clear Creek, other temperatures occur during the breeding season. Many *G. affinis* females were isolated at 10, 15, 20, 30, and 35° C. Females in 10° C water produced only two broods of dead young (one just after isolation and the other two weeks later). Females in 15° C water produced three broods of living young one, two, and three weeks after isolation. Females in 35° C usually died in one or two weeks so they could not produce second broods. Females at 20 and 30° C did reasonably well and had second broods after 60.0 and 24.0 days respectively. The small samples available (2 and 4) make precision dubious, but the dates of first broods produced after isolation (fig. 43) indicate that the pattern is correct; therefore, in early spring and late fall, broods at temperatures about 20° C occur every two months and in midsummer, around every three weeks at temperatures near 30° C.

Krumholz reported gestation intervals of 24.5 (1963, *G. manni*) and 23.9 days (1948, *G. affinis*) but did not list aquarium temperatures. If his aquaria were warm (*ca* 30° C), the data are comparable to those for *G. affinis* from Clear Creek. Maglio and Rosen (1969) reported "reproductive waves" every three to four weeks in Kalbfleisch Pond, New York and ambient temperatures about 25° C. Reproductive waves assume full synchrony of all reproductive females, and any significant deviations could result in erroneous conclusions. I am somewhat concerned by their statement (p. 11) "Young appear in waves beginning in *late May* [my italics] or early June. . . ." and (p. 25)

"The earliest recorded appearance of young was June 3, 1965. . ."; therefore, the initial dating of their sequences is questionable. If one discounts the May broods, the interbrood intervals of their fish are 31, 34, 17, and 39 days, figures not discordant with my figure of 35 days at 25° C.

Other Animals

The preserved samples contained more than 95,323 *Gambusia* from the sampling area in Clear Creek. Fewer than 1,000 other vertebrates have been contained in the same samples. They are, in descending frequency:

Dionda episcopa. A minnow with a long gut and reputed herbivorous habits. Most *Dionda* were obtained above the earth-concrete dam and transect captures were more likely in bottom traps than floating traps.

Lepomis megalotis. A few juveniles were trapped, and adults of this and five other sunfish observed in water 1-2 meters deep. The juveniles had *Gambusia* and amphipods in their stomachs.

Micropterus salmoides. A few young were trapped and adults were in open water 1-2 meters deep. The young had *Gambusia* and shrimp in their guts.

Ictalurus natalis. A few young were trapped and .many amphipods were in their guts.

Natrix sp. A dozen were trapped and those that were checked for food contents had *Gambusia, Acris,* and *Rana* in their guts.

Thamnophis sp. Four trapped (others observed) with food habits similar to *Natrix.*

Many *Rana* and *Acris* tadpoles were obtained, and adults of the latter were trapped.

The other items in traps were numerous amphipods, fresh-water shrimp, crayfish, and assorted aquatic insects.

The interactions of these animals with *Gambusia* are complex, but most are on different trophic levels than *Gambusia.* The amphipods, shrimp, and insects are mostly food for *Gambusia.* Large insects (and *Gambusia*) may eat *Gambusia,* but most energy exchange is in the other direction. *Dionda* seem to occupy a different water stratum and eat different foods. Although young *Lepomis, Micropterus,* and *Ictalurus* may eat similar foods as *Gambusia,* the piscivorous adults would act as predators.

The frequency of predators in Clear Creek is strikingly different from that in Kalbfleisch Pond (Maglio and Rosen, 1969). The absence of fish that might prey upon *Gambusia* could result in occupation of offshore (and deep) waters, whereas populations of centrarchids there might reduce that occupation. Because the mosquito control district that has been maintaining *Gambusia* in Chicago excludes all other fish from their stock ponds (during my 1969 visit *Lepomis* were first observed there and eradication procedures planned), evolution of behavioral activities in non-predated populations is likely; therefore, the offshore activities of Kalbfleisch Pond *Gambusia affinis* may reflect the condition of absence of fish predators but most *Gambusia* populations would respond like those at Clear Creek in which fish predators abound. Krumholz (1963) contrasted predated and non-predated samples of *Gambusia manni* but unfortunately did not record the inshore-offshore distribution of the fish; however, he showed that predation seemed to affect growth rate, sex ratio, and many other items. Therefore, it would be expected to influence habitats occupied.

Discussion

The small fish populations at Clear Creek are dominated by two species of *Gambusia*; an endemic, *heterochir,* and a widespread species, *affinis.* These species occupy similar trophic levels, have similar habits, extensively hybridize, and must be considered to be intense competitors. Because fish may change their food and other habits as they grow, adult stages are compared below.

Most streams in the limestone plateau of central Texas have 14-18 species of fish. Clear Creek is unique in having more *Gambusia* and fewer others (Hubbs, 1957b). A "standard" species list (the Clear Creek list follows in parentheses) would be one catostomid (0), five to six cyprinids (1), one or two ictalurids (1), one poeciliid (2), one or two percids (1), and five to six centrarchids (5). The "standard central Texas species" absent from Clear Creek collections are primarily cyprinids. Cyprinids are diverse fish and occupy many habitats in central Texas but most are midwater fish feeding on arthropods (Starrett, 1950). The only cyprinid present, *D. episcopa,* is a bottom-inhabiting herbivore or omnivore. Poeciliids are surface fish feeding primarily on arthropods, indicating that abundance of poeciliids is inversely correlated with cyprinid abundance. This hypothesis is supported by observations that the endemic *G. heterochir* is often in midwater and that its primary foods, amphipods, are rare on the surface. If *G. heterochir* were to occur in a standard central Texas stream, it would occupy a habitat similar to several species of *Notropis,* but if necessary could shift to surface habitats occupied more efficiently by *G. affinis.* The absence of *G. heterochir* elsewhere is therefore likely to involve inability for it to compete with cyprinids in one part of the environment and with *G. affinis* in another habitat in the relatively more speciose central Texas streams. The absence of cyprinids in Clear Creek is less easily explained, but central Texas spring headwaters are seldom occupied by many cyprinids (Hubbs, Kuehne, and Ball, 1953). There is no barrier to prevent cyprinid entry, therefore the absence is due to dynamic factors thought to be associated with survival difficulties of many animals in constant temperatures (Allee,

et al., 1949). Only the edges of the lower pool have fluctuating temperatures, and those areas are typically (and abundantly, at Clear Creek) inhabited by *G. affinis*. These locations are also over flocculent mud bottom, a condition unfavorable to benthic egg deposition. All areas with solid substrates also have thermal consistency. The deep waters of the lower pool would be more thermally stable than the edges. Regardless of the casual factors, cyprinids are essentially absent in the sample area which permits the two *Gambusia* to occupy a large fraction of the available habitats.

Adult darters (*Etheostoma lepidum*) have food habits similar to those of *Gambusia* but 1) they are rare at Clear Creek, and 2) they are seldom more than 2 cm above the substrate. Therefore, habitat overlap with darters is minimal.

Adult centrarchids and ictalurids are much larger than *Gambusia* and are more likely to be predators than competitors. Young have been occasionally captured, and they would compete with *Gambusia*; however, young occur only in summer and by winter (when food would be scarce) often exceed 75 mm standard length.

The other aquatic vertebrates also tend to occupy different trophic levels. Larval *Rana* and *Acris* are herbivorous; the adults would eat foods similar to *Gambusia* but usually of a more terrestrial variety. The snakes (*Natrix* and *Thamnophis*) feed on amphibians and fish; no insects or amphipods were found in their guts. The *Nutria* are herbivores. Therefore, other vertebrates present parallel fish in not occupying the same niche as *Gambusia*.

The vertebrate fauna of Clear Creek is therefore less diverse than that often found elsewhere. Although it may be coincidental, this area is also one in which extensive hybridization has been observed. Carl L. Hubbs (1955) reported many hybrid swarms in the fresh waters of western United States and few in comparable areas in eastern United States. He attributed this to the more extensive alteration of the habitats in the west. Because the aquatic fauna of western United States is depauperate, each species is unlikely to be restricted to a single narrow niche. The presence of two closely related species in a variety of environments permits some to occur together in intermediate habitats and especially any hybrid to have a niche broad enough for survival but not that to which one parental genome is specifically adapted. In a fine-grained system the intermediate niches would be nearly identical to those of the parental types.

Because most *Gambusia* species are allopatric, hybridization is unlikely. Two or more species have been found together in many relatively speciose localities in southern Texas and northeastern Mexico. Extensive hybridization has only been reported at San Marcos, Texas (Hubbs and Peden, 1969) in a thermally consistent and reasonably

speciose environment. The duration and dynamics of the San Marcos situation are unknown, but cyprinids are *not* common in the area of hybridization (Jurgens, 1951).

Gambusia in Clear Creek occupy several arthropod-consuming niches and each species is undoubtedly best adapted for one. Those individuals occupying suboptimal niches, would produce fewer young, etc. etc. The best niches of the two species are dissimilar so that intermediate niches would be suboptimal. If hybrids occupy these niches, they would displace subordinate individuals that would have minimal contribution to subsequent gene pools. Therefore, postfertilizing reinforcement (Hubbs, 1967) of isolation mechanisms would be unlikely. Circumstances in the environment reduce the probability of the operation of prefertilization mechanisms.

The existence of numerous hybrids at one locality for a prolonged interval — 1953 to 1970 — is irrefutable evidence that prefertilization (Liley, 1966) isolation mechanisms are inefficient. Isolation mechanisms have been grouped by a series of authors and the following sequence (from Liley, 1966) is best designed for poeciliid fish in which sperm release may precede fertilization for over one year.

I. Prefertilization
   a. Seasonal and habitat isolation
   b. Ethological isolation
   c. Mechanical isolation
   d. Gametic mortality
II. Postfertilization
   a. Zygote mortality
   b. Hybrid inviability
   c. Hybrid sterility

The above listing assumes that the fish are sympatric and includes most items that are known to be involved in species recognition. All of these factors are involved to a degree in the *Gambusia affinis* X *heterochir* interaction, but none works with a high degree of efficiency.

Seasonal isolation (*sensu stricto*) is likely to be inefficient in organisms with prolonged reproductive cycles. Frequently, the egg volumes produced during a breeding season exceed the volume of the female at the start of or during that period (Hubbs, Stevenson, and Peden, 1968); therefore, reproductive metabolites are being processed during the breeding season. If two sympatric species each with a 9-month breeding season were to eliminate hybridization potential by seasonal isolation, neither could have a breeding season longer than six months (one or both would have to use the adverse three months to achieve even this figure) and the reduced reproductive potential (at least by 1/3) would place those females at a serious disadvantage. Seasonal isolation is more likely in organisms that reproduce once a year. They seem to store metabo-

lites for one annual reproductive burst, and minor seasonal displacements could prohibit hybridization but not significantly lower fecundity.

Seasonal isolation does not seem to be effective in isolating *G. affinis* and *G. heterochir*. Both are reproductive from February through October (both listed months have minimal reproduction). The typical patterns differ; i.e., *affinis* has a reproductive peak in April and May and *heterochir*, in August and September; but when *affinis* is in the *heterochir* environment it has the *heterochir* fall reproductive peak. It is of course possible that *Gambusia* males court avidly and females are receptive only at specific seasons because young can be born months after courtship. Two items make this unlikely; any fish (especially males) that reduce their courtship activity would leave fewer young and consequently be at a selective disadvantage, and courtship was observed during every visit.

Differences in diel activity periods would enhance reproductive isolation. Because male *Gambusia* always seem to be courting, those that are active are courting. If females are active at the same clock hours as males, courtship is likely but is less probable if their activity periods differ. In midsummer the major morning activity peak for *G. heterochir* precedes that for *G. affinis* and morning courtships are more likely to be homospecific because of this factor.

Habitat isolation is inconsistent at Clear Creek. As discussed below, the two species have more habitat overlap in winter than in summer. Most courtship would occur in summer when the habitats are most distinct because the spring-born fish become mature then.

Ethological isolation is usually considered the most effective in poeciliid fish (Liley, 1966). Mate discrimination tests do not demonstrate strong homospecific preferences when *G. affinis* and *G. heterochir* are tested against each other (Hubbs and Delco, 1960; Peden, 1970). Although there may be low-level homospecific preferences in nature, heterospecific courtships occur in tests nearly as often as homospecific courtships. The absence of strong behavioral isolation permits extensive hybridization.

Mechanical isolation is rare (or unknown) in teleost fish. Female *affinis* have the distal segment of the genital system opening posteriorly, and female *heterochir* have that segment opening anteriorly (Peden, 1970). He was able to show that the direction of the copulatory thrust was anterior in *affinis* but posterior in *heterochir*. A heterospecific pair is therefore less likely to achieve successful copulation than a homospecific pair.

Gametic and zygotic mortality have not yet been studied in this combination, but significant survival must occur to obtain the hybrids.

Hybrid inviability and sterility are involved in reducing gene flow, but the effect depends on the habitat examined. This subject will be discussed in detail below.

It is traditional to invoke the idea of human environmental alterations in areas of extensive hybridization. An impounded stream is obviously disturbed. The undisturbed conditions at Clear Creek are difficult to reconstruct with assurance, but I have talked with several members of the Wilkinson family who own the land about conditions there prior to settlement and believe the outline below is reasonably accurate.

Clear Creek began in a series of springs at the base of and near a limestone cliff. The spring runs flowed down a gradual slope to unite in a pecan tree-lined stream that passed through the present upper dam spillway. From there the pecan-cypress tree-lined stream meandered through the area covered by the lower ponds to its crossing of Texas Highway 21. Several additional spring runs joined the creek along its course. After turning east, most or all were from the left, or north side. A slow-flowing, shaded spring-fed creek would have many of the attributes of the upper pool: constant temperature, low pH, etc., and it is likely that *heterochir* predominated throughout the creek almost to its junction with the San Saba River, three kilometers east of the source. The side springs would help maintain the spring environment along the course of Clear Creek. Following the impoundments, *G. affinis* was able to compete successfully with *G. heterochir* in a larger fraction of the creek until the latter was virtually eliminated below the upper dam.

The fauna of Clear Creek prior to impoundment is difficult to reconstruct with assurance. Only one nineteenth century collection, by W. W. Anderson, is available from the area; and Jordan and Meek's (1884) description of *Cliola urostigma* (= *Notropis venustus*) is based in part on Anderson's small collection from the San Saba River. This collection includes only *N. venustus* and *Lepisosteus osseus*, fish now common in the river at Fort McKavett. Therefore, we can assume that the fauna in the San Saba at the junction with Clear Creek has remained relatively consistent in the last 100 years. *Gambusia affinis* and several species of *Notropis* are abundant and would have had access to Clear Creek. Two cyprinids, *Notropis lutrensis* and *stramineus*, are common in the spring runs of the two prongs of the San Saba. The former, *N. lutrensis*, often spawns on gravel bottom (Hubbs and Strawn, 1956) in warm water (Minckley, in press), conditions now absent in Clear Creek. The latter, *N. stramineus*, is typically on the shallow edges of spring runs, again an environment not present in Clear Creek at this time.

When the creek was undisturbed, the contact zone between the two gambusiines was likely to have been abrupt and little intermingling would have occurred. The unnatural broad surfaces of

38

the impoundments permitted tongues of *affinis* to intermingle with *heterochir* (because of the probable changes with time it might be best to state that the most upstream individuals of *affinis* invaded *heterochir* environments in those areas most favorable to *affinis* survival). Because of the absence of pre-1953 information on the fish, it is not possible to ascertain what conditions prevailed prior to dam construction, but it is probable that hybridization followed impoundment. If so, one of the standard causal factors for hybridization, disturbed environment, is involved at Clear Creek. Of course, it is difficult to find a place in which some environmental disturbance cannot be invoked.

Another factor often involved in hybridization is relative species density (Carl L. Hubbs, 1961). The hybrids occur in the contact region between the two species. On the edges of the contact zone one of the species must be much less abundant than the other. If conditions were to stabilize and hybrids were to be absent, the relative abundances of the pure species would be of such a nature that hybridization would be likely. For example, at C1, B1, and A1, *G. affinis* is at least 10 times as likely to meet a *G. heterochir* of the other sex as a *G. affinis*. Eventually conditions would occur in which the series of isolation mechanisms would fail to maintain genetic integrity. Below the dam, conditions would be even more extreme. At localities similar to E11 or F, the ratios are closer to 100 *affinis* per *heterochir*, and somewhere near station K the ratio would be closer to 1,000 to 1. Because observations indicate that it is the female *heterochir* that enters the *affinis* environment, the following situation would be likely. A female *G. heterochir* would occur at station E9 and no male *heterochir* would be within 30 meters. The female could repeatedly reject the advances of *affinis* males, but her reproductive stimuli should increase until she was willing to succumb to the courtship of a male *G. affinis*, perhaps late in the evening when both species have activity peaks and dusk would obscure the visual discriminating cues.

Regardless of the duration of the swarm, conditions have been relatively stable at Clear Creek for more than 15 years. During the first visit on 22 February 1953, Drs. Strawn, Dobzhansky, and I collected a series of *Gambusia* by seining on the shore adjacent to and between stations B1 and C1. On 13 August 1970, Jack Waide, Darrell Yardley, and I seined the same area. Both samples contained many adult *G. heterochir*, several hybrids, and a few *G. affinis*. Therefore, conditions at that spot had not drastically changed in over 17 years. Because both sexes of *G. affinis* are able to produce at least two generations per year and few females live longer than 12 months (males probably seldom live that long), 18 to 35 generations have elapsed between seining samples

Although the pattern of hybridization has re-mained consistent for more than 17 years, some details have changed. A comparison of the species integrity shows no change that cannot be attributed to technique differences. The precise changes indicate that males are 8.6% more similar and females 8.7% more distinct in samples obtained 10-11 years later, or the extent of change is minimal. In contrast, there is a pronounced change in the amount of environmental overlap between the samples obtained in 1956-57 and those taken in 1967-68. Localities sampled both times had the dominant species more abundant in the second sample interval than in the first. The early samples had several *G. heterochir* from stations below the dam. Even during that sampling interval, fewer *G. heterochir* were found in later samples. These samples were obtained at the end of a pronounced drought that may have reduced the "*heterochir* environment" below the dam sufficiently to permit swamping by *affinis*. In summary, morphologically little change occurred but ecologically the species are more distinct. The precise causal factors for this are unknown but indicate that the endemic *G. heterochir* is likely to survive for further study.

Therefore, hybridization has occurred for at least 17 years. In addition to the generalized conditions that enhance hybrid production, some environmental factors observed at Clear Creek increase the probability of hybridization.

Studies on poeciliid ecology usually involve their occurrence at a specific time. By default any possible migrations must be overlooked. If one extrapolates from Baird's (1968) observations on *Mollienesia latipinna*, one would expect adult *Gambusia* to remain near their birth place. Absence of studies to the contrary justifies Krumholz's (1963) statement about *G. manni* "because the principal concentrations of fish were at opposite ends of the pool, it is believed that there was little intermixing", and I did not consider seasonal migrations in my previous report (1959) on population dynamics of these fish. The differential in thermal conditions reported here and in the 1959 paper shows that stations near springs are consistent thermally and that fluctuations increase with distance from springs. Because *Gambusia* is a tropical or semi-tropical genus (Rosen and Bailey, 1963), it is not likely to be adapted to extremely cold environments. It is, therefore, not surprising that *Gambusia* are most abundant at the hot (thermally fluctuating) stations in summer and at the warm (thermally stable) stations in winter. Although seasonal migrations of *G. heterochir* were not shown, the migrations of *G. affinis* could be paralleled by *G. heterochir* movements.

If we assume that at some time in the fall *Gambusia affinis* are evenly distributed in the lower pool, the following sequence of events will occur. As the season progresses, the climate cools and the air temperature has more effect on the shallow

waters remote from spring sources. Stations such as L and M become cold and *Gambusia* (especially adults) migrate offshore where temperatures are somewhat warmer. Because *Gambusia* occur in shallow water even when temperatures are equal, there is a constant stimulus for them to migrate shoreward. Whenever a warm day occurs, the fish would enter the shallows; those that by chance found a constant temperature spot would be likely to stay in that vicinity during the next cold period, but those that found another fluctuating temperature spot would return to the offshore waters when the climate cooled. Each time *Gambusia* migrated offshore, they would be vulnerable to centrarchid predation. Eventually nearly all *Gambusia* would be in constant temperature environments or eaten by centrarchids. The faster a fish found the constant temperature environment the greater the probability of survival; therefore, it is likely that such a pattern would be enhanced by any temperature sensor that permitted *Gambusia* to locate warm water (Maglio and Rosen, 1969). However, the change could involve only random movements. If seasonal changes were gradual and regular, all migration could be in shallow water; however, the climate is a series of sudden cold waves followed by gradual warming. In fall months the cooling is accentuated and the warming slowed by the overall seasonal cooling. At Clear Creek the end result would be that consistent temperature areas would receive many *Gambusia* from adjacent, and much more extensive, fluctuating temperature areas. Two types of results would occur. Regions such as the eastern spring area between stations Q and R would be subject to invasions from two directions. If all of the *Gambusia* resident in the area were *heterochir*, the invading *affinis* would vastly outnumber them. Any competition would be intensified by increased habitat similarity. During midwinter, foods would be scarce and *G. affinis* is known to use the typical *G. heterochir* food resource then. Some fish would starve, others would wander too far offshore looking for food, and any that happened to be *heterochir* would reduce the sample of the endemic species that survived the winter. Following seasonal warming, some of the *affinis* might remain and use resources usually available to *heterochir*; other *affinis* would move to the fluctuating temperature area and produce more than twice as many young as equal-sized *heterochir*. This differential would be accentuated because *affinis* females would have more young early in the year so that the F_2 *affinis* for that year would be produced earlier than the F_2 *heterochir*. The absence of *heterochir* in typical *affinis* habitats indicates a low survival potential of any *heterochir* that happened to move into fluctuating temperature environments and that their impact on *affinis* would be minimal. The *affinis* advantage may involve a winter buildup of *Gambusia*-type foods in the fluctuating temperature environment that provides optimal nutrients for the early season reproductive peak. During many summers the *heterochir* could recover from the *affinis* invasion, but if they did not, the problem would be accentuated the following winter because most of those *heterochir* would be replaced by *affinis*, which would increase the number of competitors in the *heterochir* environment. It is likely that the first visits coincided with the extermination of resident populations of *G. heterochir* below the dam. This is likely to have been accentuated by the pronounced drought and perhaps removal of fish during my studies.

A barrier such as the earth-concrete dam separating the upper and lower pools could drastically alter seasonal migrations. The *affinis* migrating toward the spring sources above the dam might frequently find suitable winter thermal environments at G, F, D1 and D10 and not be stimulated to move elsewhere. Moreover, the volume of water from the upper springs is vastly greater than that from the eastern springs which would increase the area in which *affinis* might remain without invading the center of the *heterochir* environment. Therefore, fewer *affinis* might be stimulated to try to pass through the dam. Those that did would be subject to a predation hazard. The downstream end of the break in the dam is 10 meters from the shore environment where vegetation is sparse. The predation vulnerability would depend on the time it took to move from protected inshore waters to the dam break, but few fish would be likely to escape predation. Prior to the dam break, only a few fish would enter the upper pool through the spillway. Conversely, few fish that entered the upper pool would be able to return to the fluctuating temperature environment as the climate warmed because they would have to return through the deep and predation-hazardous segment between D9 and D10.

Those few fish that entered the upper pool should cause an increase in *affinis* frequency there. Any behavioral hierarchies that had been set up among *affinis* would be absent because so few invaders would remain that the presence of recognized ranked individuals would be unlikely. In the area adjacent to the dam break (and perhaps even in the stream) and spillway, *affinis* populations would be established. Any such population would be increased annually in winter and provide a store of *affinis* to supply individuals for the upper pool. Hybridization could occur between fish in or adjacent to those *affinis* "beachhead areas."

During the preceding summer reproductive season, the populations of *G. heterochir* would have increased. The upper pool would be filled with fish. Many *heterochir* would be unable to find optimal environments and enter suboptimal areas, and in doing so some would migrate through the dam. The intensity of the crowding would be just after the *heterochir* breeding peak or in early win-

ter when the *affinis* were entering constant thermal environments. It is probable that on occasion those *heterochir* remaining in the dam break would be isolated with *affinis* migrating through that same spot. If they were of opposite sexes, hybridization possibilities would be enhanced. This circumstance would be relatively infrequent because most evidence indicates that females are more likely to move than males. All *heterochir* found below the dam are female. The major migrations below the dam emphasize large (= female) individuals. The probable causal factors are thought to involve male territoriality and female schooling. Even if infrequent, heterospecific opposite sex synchrony in the dam break would increase the potentiality of producing hybrids.

The population structure in the upper pond is such that hybridization potential is enhanced. *Gambusia* are unevenly distributed. In areas of dense populations, most individuals are *heterochir*; therefore, a standard hybridization situation prevails, uneven numbers of fish so that the rare type may lose its discrimination. A greater potential for hybridization occurs in the sparsely populated areas. In these regions, the two species may be in nearly equal numbers, but the males are typically *heterochir* and the females typically *affinis*. If only two adults are present and they are of opposite sex, about one-half of the instances will, in effect, be non-choice mating tests. Because these conditions are most pronounced in the least densely populated areas, the possibility of choice is virtually eliminated. The species-sex distribution results from a combination of migratory activity and dominance hierarchies. When *Gambusia affinis* enter the upper pond in winter, they invade an area saturated with *Gambusia*. Strange (= invading) fish first occupy low levels in the dominance hierarchy and would consequently be forced to suboptimal habitats (Baird, 1968), and the more highly territorial males are less likely to enter the upper pool. Therefore, the *Gambusia affinis* in the upper pool are most often subordinate females. After adjusting to upper pool conditions, the *Gambusia affinis* could occupy higher positions in the hierarchy. The sites of invasion would also be those with very dense *Gambusia* populations. As such, population pressures would force emigration from the southeastern corner of the pond. The fish can only go north or west (except for the few that might enter the lower pool) where adjacent waters have sparse populations of *Gambusia*. Therefore, many of the invading *Gambusia affinis* (primarily females) would be located in sparsely populated, suboptimal environments in the upper pool. *Gambusia* males are known to form dominance hierarchies in aquaria (McAlister, 1958) that probably operate in nature similar to those of *Mollienesia* (Baird, 1968) in which small males are omega and most commonly in suboptimal environments. Such dominance hier-

archies should be present in *heterochir* populations so that subordinate males would be forced into suboptimal environments. These are the same localities which the *affinis* females would be predicted to occupy. In sum, the collection of *heterochir* males more or less isolated with *affinis* females has a logical explanation and would make hybridization almost inevitable.

Hybrids have been known from Clear Creek for at least 17 years (= up to 35 generations), and on each visit the frequency was similar. Hybrid females have a fecundity intermediate to those of the parental species as do apparent backcross hybrids. If the factors associated with production of F_1 hybrids were to apply equally well to production of young by hybrids, the two species should have fused in much less than 17 generations. The maintenance of *G. heterochir* in the presence of *affinis* and hybrids in its entire geographic range is irrefutable proof that the isolation mechanisms are effective. (The presence of *G. affinis* in the area could merely result from immigration from pure *affinis* populations in the lower pond.) The distribution of male hybrid index codes is highly suggestive of problems of introgression. Both analyses show that the male distribution is trimodal; i.e., many *affinis, heterochir,* and intermediate types are found but few adult males seem to represent backcrosses, all of which indicates that postfertilization isolation mechanisms are more significant than prefertilization mechanisms. It is also obvious that no postfertilization mechanism is absolute but all work together to reduce or eliminate the impact of introgression. In sum, the genome of each species is adapted to survival in a specific habitat and in the presence of the rest of the genome of that species. The individual discordancies are hard to find, but some indications are available from the field observations.

The niches occupied by *G. affinis* and *G. heterochir* differ in details, and the differences seem to be maintained when they occur together in the upper pool. *Gambusia heterochir* has an early morning activity peak and *G. affinis* is active in mid-morning; above the dam the *G. affinis* are also most active in mid-morning. *Gambusia heterochir* can be observed to swim around and among clumps of *Ceratophyllum* just below the surface, and *G. affinis* seldom leave the surface; perhaps as a result, *G. heterochir* seems to use amphipods as a primary food source and *G. affinis* often has insects (many of terrestrial or surface origin) in their guts. This food preference is maintained in the upper pool where the two species occur together. All of these items indicate that each species is an integrated whole and that foreign chromatin might upset the delicate balance that would be expected to be most favorable to survival.

The virtual absence of *G. heterochir* from collections obtained below the earth-concrete dam

is reasonable evidence that *G. heterochir* is poorly adapted for that habitat. The absence could be due to inability to survive in fluctuating temperatures and the associated biological and chemical factors or the inability to compete with *G. affinis* in this type of environment. Because *G. heterochir* is rare below the dam in thermally consistent environments that appear to be equivalent to those above the dam that are filled with *G. heterochir,* competition is indicated to be a major causal factor. We further tested this hypothesis with artificial populations in 3-m diameter by 0.7-m-deep plastic pools. The pure *G. heterochir* population was difficult to establish but then survived one year at ambient temperature. Five mixed populations at ambient temperature were pure *affinis* six months later and each of the five with thermal extremes reduced by a flow of well water, had a few *G. heterochir* after six months. These results suggest that competition is the critical factor even when the environment is favorable.

Because *G. heterochir* is seldom or never found in the absence of *G. affinis*, it is not possible to contrast sympatric and allopatric field samples of *G. heterochir*. In contrast, the distribution of *affinis* is amenable to such comparisons. If we assume that factors associated with survival also are associated with growth rate and relative fecundity, we can make predictions on survival potential. Such comparisons do exclude predation but, with that exception, should provide reasonable evidence of adaptations to those environmental conditions. The growth rate of *G. affinis* samples obtained from the consistent thermal environments is distinctly lower than those from adjacent fluctuating temperature locations. Between April and July, the overwintering fish grow 4 mm at constant temperatures and at least 7 mm at fluctuating temperatures, and the newborn grow 13 and 15 mm respectively. In a similar fashion, the fecundity of equal-sized females reflects better conditions in thermally variable environments. The average female at fluctuating temperature stations will produce 1.49 young per brood for every young produced at consistent temperature stations. Both of these factors apply to females during April and May when the average temperatures in the two areas are comparable. Therefore, if we assume that two 24-mm females are together near the dam in February and one remains and the other migrates, the one that migrates will produce 3.38 times as many young per brood in July (and during the remainder of the breeding season) due to growth and fecundity differentials. During warm months, the fecundity comparisons will be even more extreme because broods are produced at about 60-day intervals at 20° C and after about 24 days at 30° C so that the female that migrates will have about seven times the reproductive potential of her sister that remains in the winter environment. In addition, the young from non-migratory females will become reproductive later and produce fewer F_2's. Females returning to the thermally fluctuating environment during March and April would be likely to remain inshore because each successive sudden cold wave is likely to be warmer than the preceding one so that extreme cold is unlikely in an onshore migratory route. The selective advantage for *G. affinis* to live in the thermally fluctuating environment is pronounced, and any fish that could sense the environmental gradient would have an advantage over those that could not.

Comparisons of adaptability of *G. affinis* in equivalent environments on either side of the dam are limited to fecundity studies because growth calculations could not be made above the dam. It is probable that growth phenomena would show equivalent changes and is unlikely that growth data would reverse fecundity data. *Gambusia affinis* in an equivalent environment — but in the presence of a competitor — have a fecundity that is about 93% of those in the absence of the competitor, showing that *G. affinis* is at an extreme disadvantage in the upper pool. It is likely that immigration is required to maintain the *G. affinis* populations above the dam. The difference in fecundity of *G. affinis*-type hybrids on the two sides of the dam is even more extreme. If we assume that the environments on the two sides of the dam are equivalent, *Gambusia heterochir* would be able to compete here better than *G. affinis*; however, swamping due to winter migrations would have essentially eliminated *G. heterochir*. Those few *G. heterochir* below the dam would seldom meet adults other than *G. affinis*, and their offspring would be hybrids which in turn would also seldom meet *Gambusia* adults other than *G. affinis*; these offspring would be the *G. affinis*-type hybrids. Because of the scarcity of any fish more like *G. heterochir* (or better adapted for the constant temperature environment), the *G. affinis*-type hybrids would have a selective advantage over the other *Gambusia* that were in the area, and females should produce many eggs. In contrast, similar fish above the dam would have to compete with dense populations of *G. heterochir* and should produce few eggs as was shown in the samples obtained.

Superficially, comparisons of fecundity of females in the upper pool at Clear Creek indicate that *affinis* is favored over *heterochir*. For every *heterochir* egg produced, an equally long *affinis* produces 1.31 eggs. The problem is increased by incubation period differences; i.e., for every *heterochir* brood, *affinis* females produce 1.20 broods. Therefore, on a purely numerical basis, *affinis* females produce 1.57 young per young produced by equivalent *heterochir* females. Those calculations ignore standard errors so the precise figure may deviate somewhat from 1.57, but the pattern is valid. In contrast, newborn young *heterochir* are

9 mm and newborn *affinis*, 7 mm standard length. This difference excludes the fact that newborn *G. heterochir* are relatively deeper-bodied than newborn *G. affinis*. This difference is reflected in the average diameter of mature eggs in early developmental stages (1.42 for *G. affinis* and 1.75 for *G. heterochir*) in the September 1967 preserved sample. The best figures are those of volumes and caloric content of comparable eggs and embryos in the August 1970 sample. If the egg volume and caloric content values are incorporated into the fecundity comparisons, the real fecundity of *heterochir* above the dam is 1.43 and 1.23 times that of *G. affinis* there. The selective advantage of *G. heterochir* in the area above the dam is distinctly greater than that of *G. affinis*.

The fecundity differences between *G. affinis* and *G. heterochir* fit the classic patterns of quantity *vs.* quality (Lack, 1954; Svärdson, 1949) even though both produce vigorous well-developed young. Environmental parameters vary extensively in the typical *G. affinis* environment; therefore, physically caused catastrophes are likely and, if not, the associated migrations could result in severe predation. Therefore, the populations are likely to have large fluctuations, and rapid recruitment of young would be favorable. The physical changes are minimal above the dam and survival potential is high. Young would seldom or never be born there in a catastrophically depleted population; therefore, those young with the most nutrients would have the greatest survival potential. A contrast of the eurythermal conditions at Clear Creek with those in Chicago provides a similar comparison. Winter physical conditions in Chicago are exceedingly severe and heavy mortality may occur; the fecundity of Chicago *Gambusia affinis* is strikingly greater than that of the same fish from Clear Creek.

Despite the presence of abundant hybrids for more than one decade, each species has maintained its distinct morphology, showing that each is a distinct and integrated gene pool. The factors influencing genetic divergences are at least as great as those which might cause genetic fusion.

Summary

An analysis of more than 95,323 preserved and 1,028 live *Gambusia* obtained data on several aspects of life history in addition to those on hybridization and interspecies interactions. The tests with living fish were in agreement with the data obtained from preserved samples.

The most dense populations of *Gambusia* were in shallow water with thick aquatic vegetation. These areas also contained the largest fish. Fish abundance and size typically decreased with distance from the optimal areas and with dissimilarity to those conditions. Males were more evenly distributed than females; consequently, more reproductive females were in the vicinity of the large males in the shallow water with dense vegetation. Those environments with few and small fish and with nearly equal sex ratios are considered to be suboptimal *Gambusia* habitats.

Gambusia trap captures were distinctly greater during some clock hours than during others. Traps set during darkness had few fish as did those set during early afternoon hours. Morning and late evening traps contained the most fish. The degree of diel periodicity was size-correlated with small fish having more nearly equal activity than large fish. *Gambusia affinis* has one morning activity peak (around 11 A.M. Central Daylight time), but *G. heterochir* is more likely to be active around 0700 and 1300. Both species are active at 1900.

Gambusia females grow rapidly until sexual maturity, after which growth slows. Mature females may grow in spring months, especially when in a thermally fluctuating environment. Females born in early spring are sexually mature by midsummer and all females are mature in early spring, showing that at least two generations can occur annually. Males do not grow after becoming mature, and midsummer males are much smaller than those in other seasons, again showing two generations per year.

The natural stock of *Gambusia heterochir* is restricted to a small impoundment on the headwaters of Clear Creek. Most of the hybrids are in the upper impoundment adjacent to a break in the dam through which *G. affinis* can enter the "typical *G. heterochir*" environment. The areas with large numbers of *G. heterochir* have a different amphipod and *Ceratophyllum*, lower pH, and a more consistent temperature from those in which *G. affinis* is most abundant. The morphological distinctions between *G. affinis* and *G. heterochir* have not been appreciably reduced despite the constant presence of large numbers of hybrids for more than 17 years. The ecological distinctions between the two parental taxa seem to have become significantly greater in slightly more than 10 years. Both species of *Gambusia* seem to prefer arthropods, but *G. heterochir* has more amphipods and *G. affinis* more insects in their stomachs. The differences are most pronounced in summer but in winter when food is scarce, the dietary items are most similar. Few potential food competitors from other fish taxa occur in Clear Creek, providing the possibility of available ecologic niches for hybrids between those to which the parental taxa are specifically adapted.

Populations of *Gambusia affinis* are most abundant in fluctuating temperature environments in summer and in constant temperature environments in winter. This results in a fall and winter migration of *G. affinis* toward and into those areas occupied by *G. heterochir*. At about the same season the population densities of *G. heterochir* are most

dense and the excess seem to move toward the *G. affinis* environment. Because females seem more prone to migrate than males, females are likely to be isolated from homospecific males. Those adults in sparsely populated (suboptimal) sites in the upper pool (typically occupied by *G. heterochir*) have 70% of the males *G. heterochir* and 70% of the females *G. affinis*. These factors increase the potentiality of F_1 hybrid production.

All analyses of male morphology produce a trimodal distribution indicating a higher frequency of F_1 hybrids than backcrosses, yet putative hybrid females have a fecundity equivalent to the parental types. The low survival of backcross hybrids and of potential introgressive genetic material may result from inability to compete for a niche in the presence of a co-adapted species gene pool. The severe reduction in fecundity of hybrids in the upper pool in which both species are common contrasts with high fecundity of hybrids in the lower pool in which *G. heterochir* is essentially absent. Fecundity contrasts also show why *G. affinis* does not replace *G. heterochir* in the upper pool. Equivalent-sized females have many more eggs if in the fluctuating temperature environment in the lower pool, many fewer eggs in the consistent temperature environment in the lower pool, and even fewer eggs in the consistent temperature of the upper pool where *G. heterochir* predominates. In addition, *G. affinis* in the constant temperature environments have a lower growth rate than those in the fluctuating environment. Both factors reflect an adaptation to fluctuating temperatures by *G. affinis* because they can be observed on data obtained when the average temperatures are comparable.

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