TEMPORAL CHANGES IN THE *Gambusia heterochir* X *G. affinis* HYBRID SWARM FOLLOWING DAM RECONSTRUCTION

by

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Published by

U.S. Fish and Wildlife Service
P.O. Box 1306
Albuquerque, New Mexico 87103

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Abstract

The long occurring _Gambusia_ hybrid swarm in Clear Creek, Menard County, Texas, involving the Endangered endemic _Gambusia heterochir_ and G. _affinis_ was monitored quarterly through the winter season of 1983-84 following the repair and reconstruction of the uppermost dam on Clear Creek in 1979.

The repaired dam now prevents the immigration of G. _affinis_ into the upper spring pool inhabited by G. _heterochir._ As a result, there has been a dramatic decrease in the incidence of hybridization in the upper spring pool as well as a moderate decrease in G. _affinis_ abundance in this upstream environment.

Downstream populations of these two _Gambusia_ species appear to be hybridizing with greater frequency than in the upper spring pool, although at levels considered less than in previous decades. The introduction of the coastal cyprinodontid, _Lucania parva,_ in the portion of Clear Creek below the upper dam may be responsible for the observed increase in abundance of G. _heterochir_ at downstream spring-fed stations, presumably by interference competition with G. _affinis._

Figure Legends:

Figure 1. Map of Clear Creek, Menard County, Texas.

Figure 2. Map of Clear Creek headspring pool showing percentage of adult Gambusia collected in 1968-69 that were **G.** heterochir.

Figure 3. Map of Clear Creek headsprings showing location of upper dam and collection localities. Numbers above and below dam indicate locations of minnow trap sets. R refers to the location of the seining locality, Station R.

Figure 4. Relative abundances of Gambusia heterochir, **G.** affinis and hybrids taken in minnow traps at stations above the upper dam. Data from 1956-57 (Hubbs 1959) and 1968-69 (Hubbs 1971) are shown for comparison.

Figure 5. Relative abundances of Gambusia heterochir, **G.** affinis and hybrids taken in minnow traps at stations below the upper dam. Data from 1956-57 (Hubbs 1959) and 1968-69 (hubbs 1971) are shown for comparison.

Figure 6. Relative abundances of **Gambusia** heterochir, **G.** affinis and hybrids taken by seine at downstream stenothermal springs, Station R. Data from 1956-57 (Hubbs 1959) and 1968-69 (Hubbs 1971) are shown for comparison.

Figure 7. Changes in relative abundances of hybrid Gambusia taken at stations above the upper dam, below the dam and at Station R at Clear Creek during the 1980-84 sampling period. Percentages are reported as the percentage of all Gambusia that are represented by hybrid individuals in each environment in the Clear Creek collections.

Figure 8. Numbers of Lucania parva taken in each season in Clear Creek collections below the upper dam and at Station R. No Lucania were taken above the upper dam. Lucania in Below Dam collections were taken in minnow traps, those at Station R were collected with seines.

TABLES

Introduction

The Clear Creek gambusia, Gambusia heterochir Hubbs, is restricted to the spring-fed headwaters of Clear Creek (Menard County), a tributary of the San Saba River in west central Texas (Fig. 1). Approximately 100 *years* ago, the first of three impoundments along the springrun was built and by the time of the initial discovery of this species in 1953, the upper dam had begun to deteriorate (Hubbs 1957, 1959).

With the construction of the dams, the only known habitat of the Clear Creek gambusia was extensively modified from a spring run to a headspring stenothermal pool above the upper dam and below this, a shallow eurythermal pool extending several kilometers. In this downstream impounded section, an extensive population of the mosquitofish, G. affinis, became established, while nearly all of the G. heterochir population occurred above the upper dam. An apparently stable hybrid swarm between these two species occurs in the shallow waters along the edges of the dam. This hybrid swarm has been in existence since the first recorded collections were made during the 1950s. There are a number of differences of ecological importance in the environments above and below the dam including differences in pH (6.2-6.7 above and 6.8-7.6 below), aquatic macrophytes (Ceratophyllum above and Myriophyllum below) and amphipod species (the endemic Hyalella texana above and the widespread H. azteca below). The correlation between H. texana and G. heterochir was not unity, however, as H. texana (but not G. heterochir) was also found in the few stenothermal springfed areas below the upper dam in addition to in the upper pool (Hubbs 1959, Stevenson and Peden 1973).

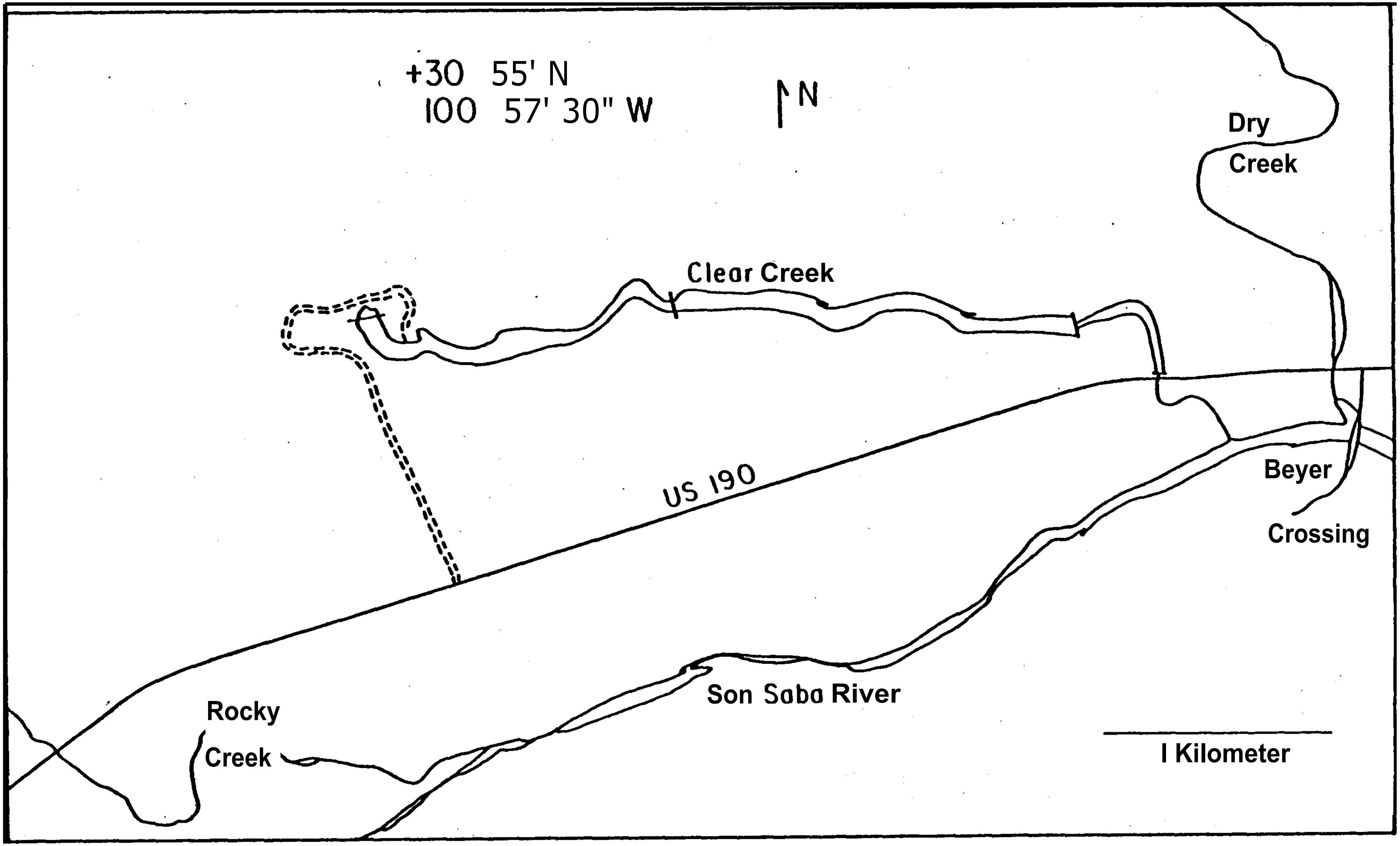

Figure 1. Map of Clear Creek, Menard County, Texas.

Extensive ecological studies in 1968-69 (Hubbs 1971) determined that the most likely source for the observed hybrids resulted from the immigration of female G. affinis through the damaged portions of the dam, especially along the eastern-most section into the upper pool. Presumably naive female G. affinis would mate with subdominant G. heterochir males, resulting in the production of F1 hybrids. The frequency of G. heterochir in the upper pool is greatest away from the damaged section along the eastern end of the dam and away from the center of the upper pool where hybridization was hypothesized to occur (Fig. 2). This was confirmed by the reproductive studies of Hubbs and Reynolds (1957), Warburton et al. (1957), Hubbs and Delco (1960) and Peden (1973, 1975) who have shown that while a partial mechanical isolating mechanism exists between G. heterochir and G. affinis, there is only a low level preference for conspecifio mates allowing the potential for hybridization. Evidence of temporal differences in the activity patterns (Hubbs 1971) of the two species showed that the probability of hybridization is at least partially lessened by differences between G. heterochir and G. affinis in their times of maximal activity.

At most localities in the hybrid zone, the hybrids had consistently accounted for more than 10% of the Gambusia present. Since G. heterochir was absent (or nearly so) in areas below the upper dam, the incidence of hybridization downstream was far less than in the area immediately above the dam (Hubbs 1959, 1971; Yardley and Hubbs 1976).

Because of G. heterochir's very limited distribution and because of the potential for genetic swamping and competition with G. affinis populations, the Clear Creek gambusia was listed as an Endangered species by the U.S. Department of the Interior in 1967 (U.S. Fish and Wildlife Service 1980).

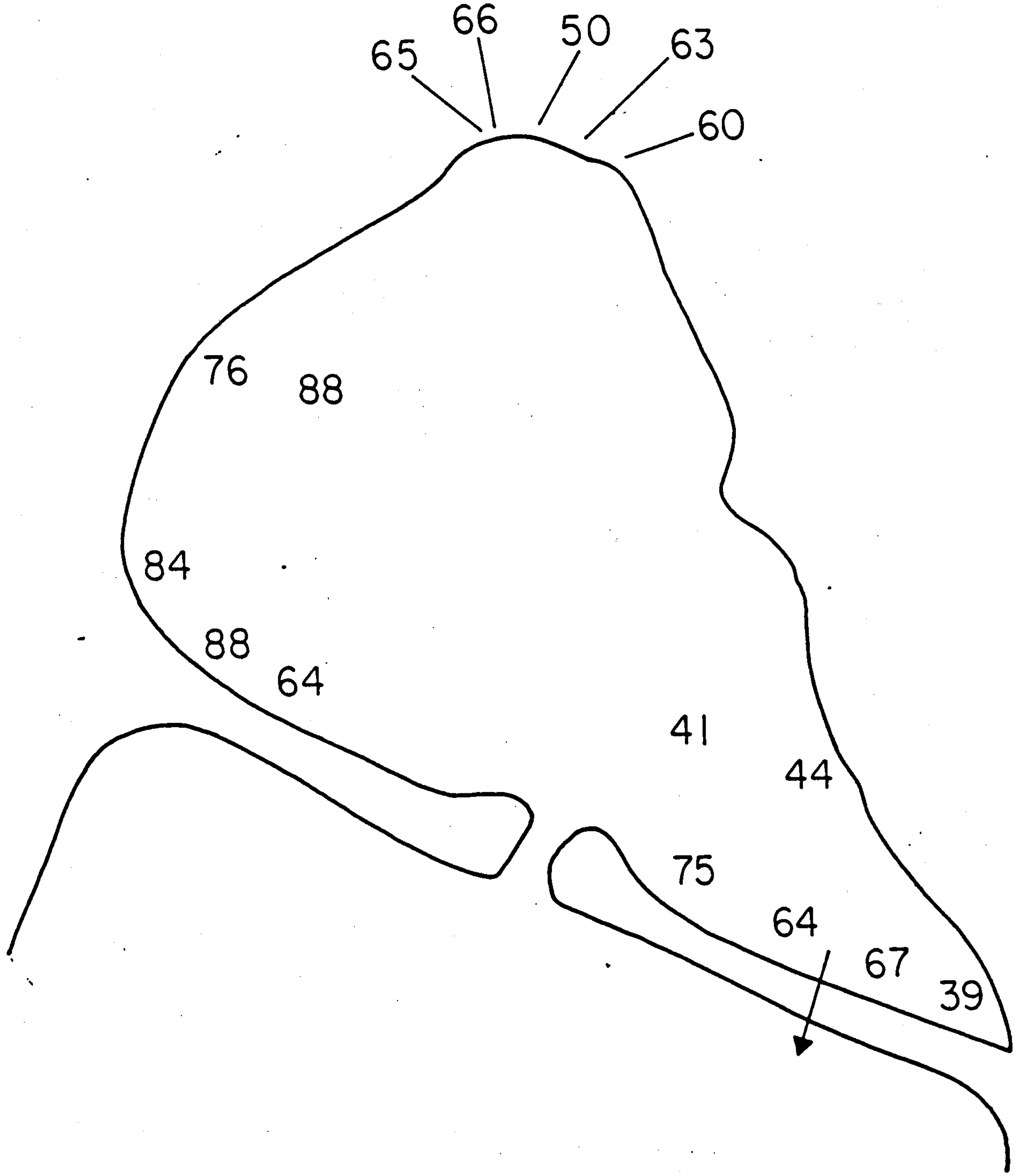

Figure 2. Map of Clear Creek headspring pool showing percentage of adult <u>Gambusia</u> collected in 1968-69 that were G. <u>heterochir.</u>

During a periodic monitoring of the status of G. heterochir in 1978, it was noted that the dam had deteriorated to the point where there were few restrictions to the upstream immigration by G. affinis into the G. heterochir environment above the dam. The dam was originally constructed across Clear Creek by means of two limestone block walls filled with earth but without using mortar. As the dam averaged less than two meters wide with elevations in excess of three meters over much of its approximately 100 meter length, minor slumping had resulted in areas where G. affinis could enter the headspring pool of Clear Creek. This problem was exacerbated by the damage from large trees on the dam (strong winds had uprooted at least three) which together with numerous tunnels dug by nutria that had been introduced into Menard County in the 1940s had considerably weakened the dam. In addition to the stream flowing through the major break in the dam near the east end, two 8 meter sections of the lower retaining wall on the west end had completely collapsed into the lower pool allowing water to leak through. Also, the spring below the dam at the east end had seriously eroded the eastern terminus of the dam and was flowing from between the two limestone block walls. At this location, only the upper wall separated the two pools. It was decided that repair of the dam was necessary to prevent massive contamination of the upper pool by G. affinis. The dam was rebuilt 27 August - 1 September 1979. All woody vegetation was removed from the dam crest. The collapsed walls were replaced with mortared limestone and the most threatened sections filled with concrete. Sand and gravel supplemented with limestone rocks from adjacent ranch lands were used to level the top of the dam. On all visits subsequent to 1 September 1979, all holes in the earth fill (presumably dug by nutria) have been filled with pieces of limestone. During the reconstruction of the dam, the wooden boards in the dam gate were removed (and later replaced with new boards) to reduce springhead water pressure while pouring concrete into weak sections of the dam through which water was seriously seeping. Some endemic animals were observed washing into the lower pool along with vegetation that was also seen to pass through the dam gate. After finishing the reconstruction of the dam, new gate boards were installed creating a drop in elevation at the spillway of approximately one decimeter. This drop is considered sufficient to preclude upstream migration by G. affinis. Following the reconstruction of the dam, dye was placed upstream at the site of the former broken areas to check for leakage; none was found. On later visits, minor flows through the dam have been ascertained; none are deemed sufficient to permit upstream migration by Gambusia (or Lucania).

The purpose of this study is to document the status of G. heterochir and the hybrid swarm once upstream immigration by G. affinis was halted and to further document the long-standing nature of this apparently stable hybrid swarm.

Materials and Methods

Samples of Gambusia were obtained by modified minnow traps at quarterly intervals beginning 26 January 1980. Plastic minnow traps were adapted for taking very small Gambusia by the addition to the outside surfaces of fine nylon screening held on with silicon sealant and reinforced with thread. Traps were placed at 16 locations along and adjacent to the newly repaired dam (Fig. 3). As the suspected location of G. affinis immigration into the upper spring pool had been blocked, the sampling efforts were concentrated along the east end of the dam to determine the effects of the immigration deterrent. The 8 upper stations (two traps each) were matched with 8 lower stations (one trap each). One trap was set at the westernmost end of the dam, 5 sets along the eastern

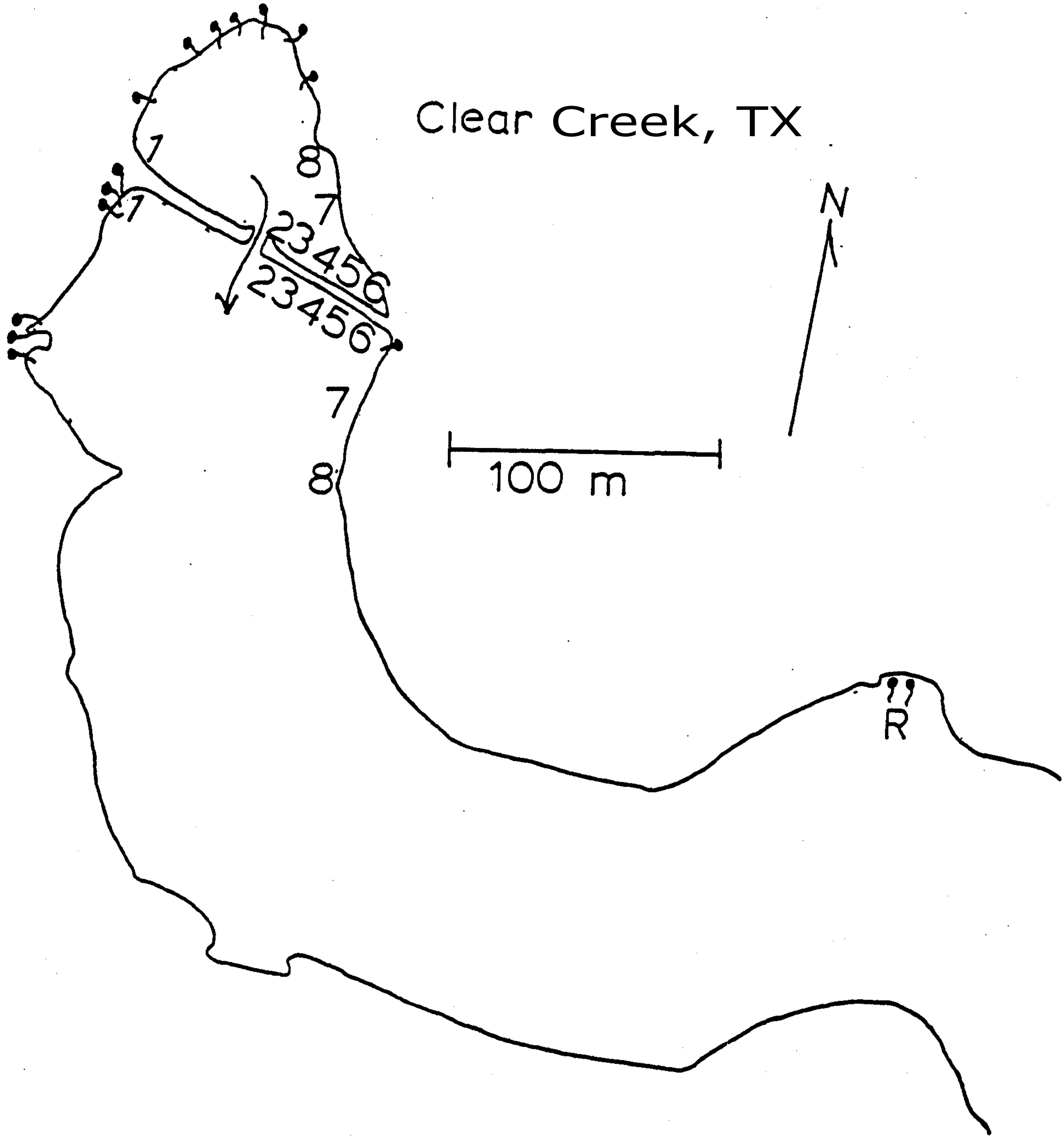

Figure 3. Map of Clear Creek headsprings showing location of upper dam and collection localities. Numbers above and below dam indicate locations of minnow trap sets. R refers to the location of the seining locality, Station R.

side spaced equidistant from the spillway gate to the shore and two additional sets of traps along the eastern shore. The former largest break in the dam was between trapping stations 4-5 and the eastern shore stations were at the same place as the 1968-69 trap stations b-10 and b-9 (above the dam) and e-11 and e-9 (below the dam). After discovering that the rainwater killifish, Lucania parva, was present in the lower pool, a seining station (Station R) was established at the downstream series of springs beginning on 25 October 1980. This station is approximately the same as the 1958-59 station D and is in the same location as the 1 968-69 station R.

The method of trapping was similar to the protocol established in 1968-69. The traps were baited with Ken-l Ration dog food, placed in the water at the trap locations at approximately 0800 hours, checked for fish at approximately 1100 hours and removed at approximately 1400 hours (Central Standard or Central Daylight Time as applicable). Captured fish were preserved in a 10% formalin solution and allocated to species or hybrid by the hybrid index code described and used by Hubbs (1959, 1971). Because this set of discriminating morphological and meristic characteristics is only valid for adult Gambusia, juveniles were judged as G. heterochir, G. affinis or hybrids by visual inspection. Similar to the 1968-69 study using minnow traps, the vast majority of the individuals taken were Gambusia (N=25,157). A total of 1855 vertebrates in addition to the Gambusia were taken. Only Dionda episcopa (N=587) and a single Ictalurus natalis were obtained above the dam and only Lucania (74), Dionda (2) and Lepomis megalotis (9) were obtained in the traps below the dam. Ictalurus natalis (2), Lucania (1151) and Lepomis megalotis (9) were taken in the seining samples. Despite their absence from our samples, numerous Micropterus salmoides and various other Lepomis were observed or captured by hook and line on each visit. The only other vertebrates captured were Rana tadpoles; 18 were taken in traps above the dam and 2 additional individuals were taken from the Station R seining locality. Numerous Nerodia and nutria were observed on summer visits.

Following the methods of Hubbs (1957) hybrid index coded fish are mature males with clear gonopodial tips and females more than 26 mm standard length (SL). All others are categorized as juveniles even though it is known that some females as small as 16 mm SL may produce a small number of offspring (Hubbs 1971). As defined by Hubbs (1971) adult male G. affinis had hybrid code values of less than 59, G. heterochir have values of 83 or higher, hybrids between 66 and 75 and various **backcross** combinations have values intermediate to these. In adult females, G. affinis have index codes of 80 or less, G. heterochir have values above 95, hybrids are between 86 and 90 while the various **backcross** combinations result in intermediate values.

Results

Following the reconstruction of the dam the abundance of G. heterochir, G. affinis and the hybrid swarm varied seasonally, as occurred in previous studies (Table 1). At the trapping stations above the dam, the abundance of G. heterochir, relative to the other Gambusia collected rose from approximately 70% in 1980 (averaged over the year) to about 95% at the end of the sampling period (Fig. 4). The initial levels of G. heterochir after the dam repair were similar to the levels found in the 1968-69 study (71% of all Gambusia trapped above the dam). The abundance of the hybrids and G. affinis steadily declined during 1980-1983. During the prior studies, hybrids were about 10% of the Gambusia captured. Shortly after the reconstruction of the dam, hybrids averaged about 5% of the Gambusia taken and by 1983 had decreased in abundance about four-fold

Table 1. Numbers of *Gambusia* taken in traps above reconstructed dam, in traps below the dam and by seining at Station R for each collection date.

DATE	ABOVE **DAM**	BELOW **DAM**	STATION R
1/26/80	427	75	
4/19/80	402	3	
8/23/80	712	147	
10/25/80	683	83	363
1/17/81	299	59	244
4/18/81	586	24	373
8/5/81	1895	278	324
10/24/81	1668	220	406
1/30/82	504	83	299
5/1/82	780	30	286
8/21/82	1552	294	890
10/30/82	1434	177	543
2/5/83	612	10	484
5/14/83	637	28	186
8/13/83	1667	286	370
11/5/83	2252	383	542
2/11/84	1036	206	315

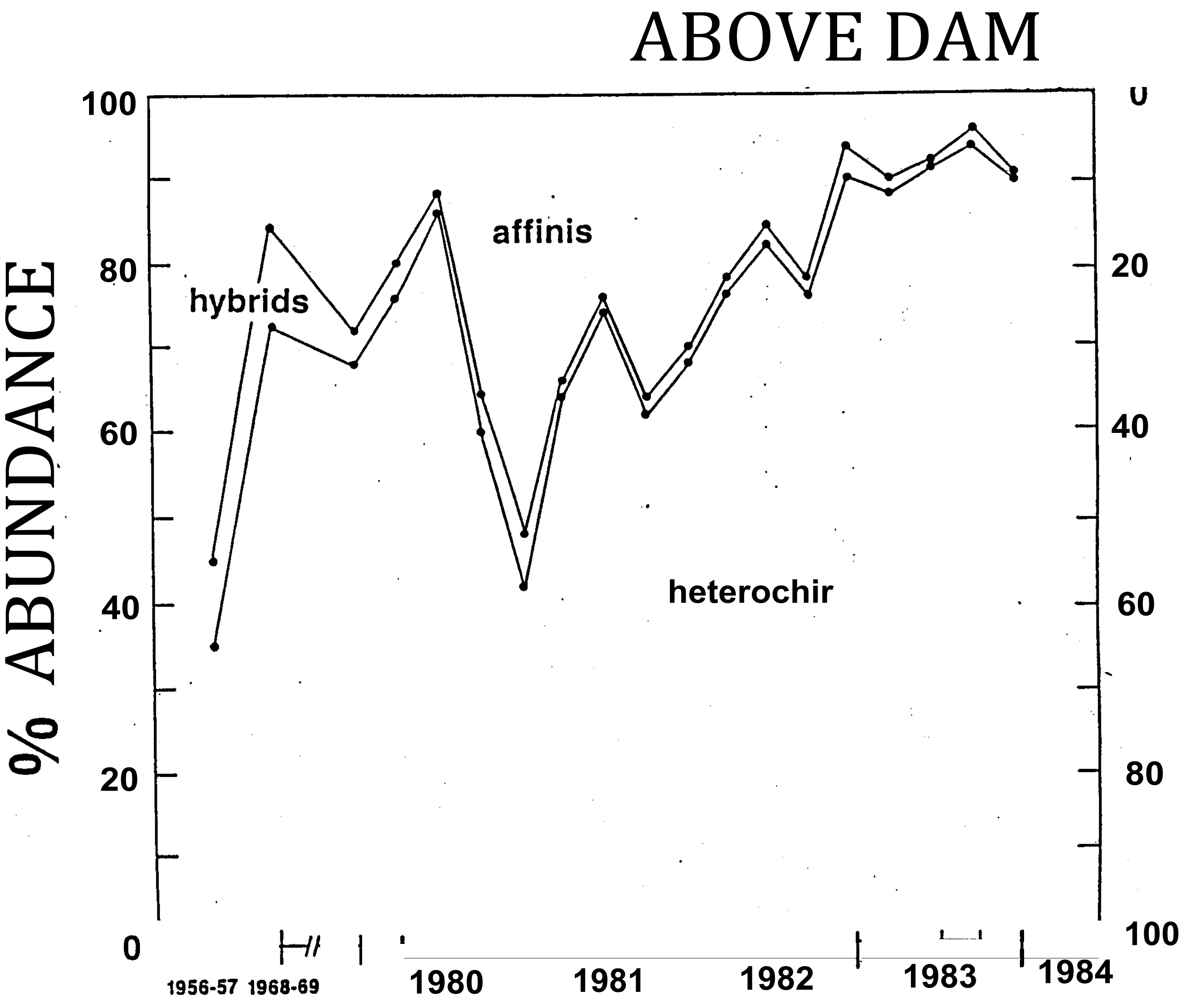

Figure 4. Relative abundances of <u>Gambusia heterochir</u>, G. <u>affinis</u> and hybrids taken in minnow traps at stations above the upper dam. Data from 1956-57 (Hubbs 1959) and 1968-69 (Hubbs 1971) are shown for comparison.

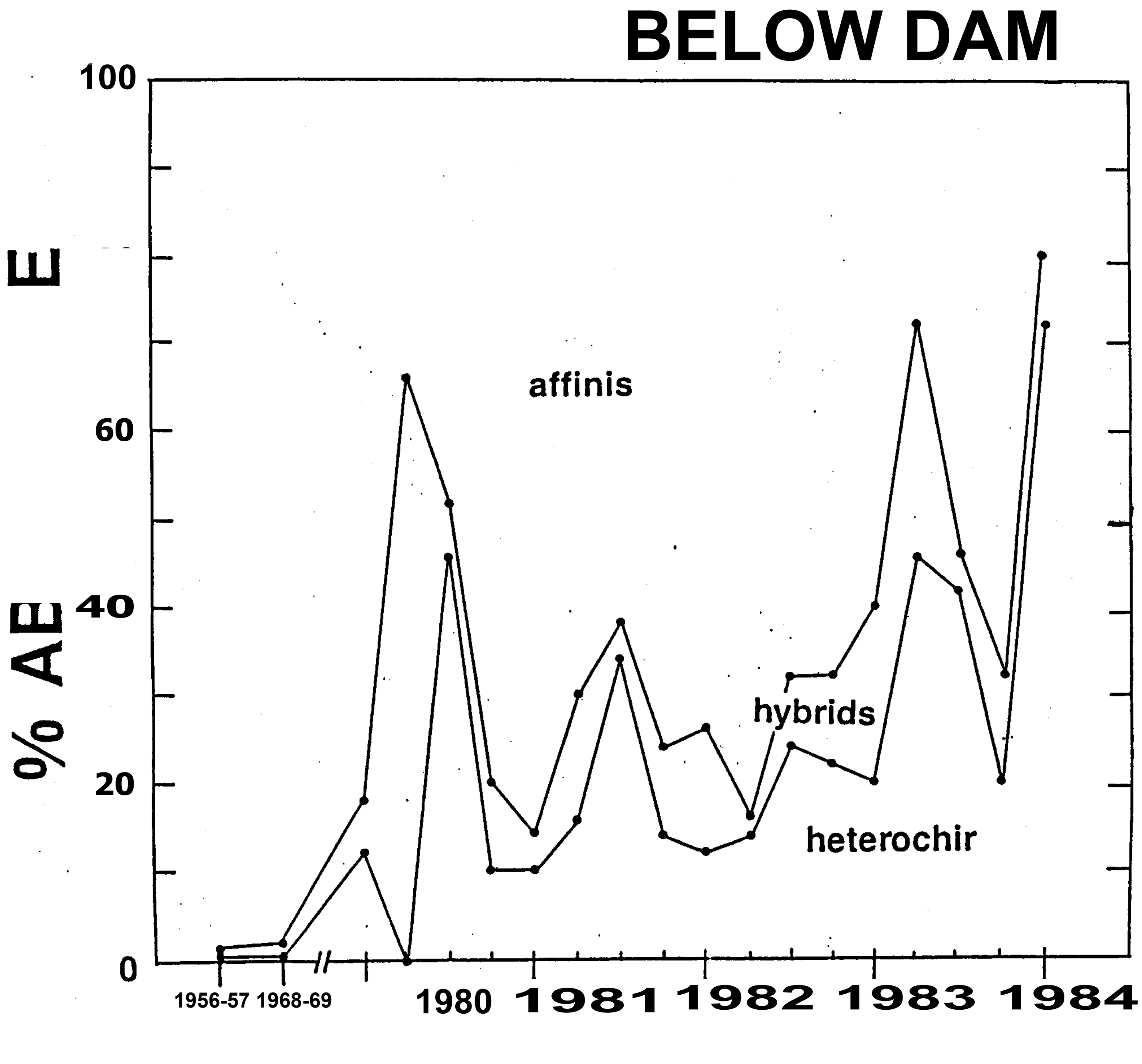

Figure 5. Relative abundances of Gambusia heterochir, G. affinis and hybrids taken in minnow traps at stations below the upper dam. Data from 1956-57 (Hubbs 1959) and 1968-69 (hubbs 1971) are shown for comparison.

(Fig. 7). _Gambusia affinis_ populations similarly declined during this period, accounting for almost 35% of the _Gambusia_ present in 1980, but less than 10% in 1983. In both _Gambusia_ species and their hybrids taken at the trapping stations above the dam, significant :seasonal fluctuations were apparent. In general, highest relative abundances of G. _heterochir_ were recorded in summer months and lowest levels in cool months. In G. _affinis_ and the hybrids, this seasonal pattern appeared reversed; highest abundance levels were consistently found during winter and lower relative abundances were found during summer collections. The magnitude of the variation in seasonal _Gambusia_ abundance also varied over the period of investigation. During the first year after dam repair, the abundances of G. _heterochir_ and G. _affinis_ changed greatly, by a difference of as much as 40%, indicating great variation in species abundance. However, the seasonal fluctuations in abundance were greatly dampened with time, such that the change in seasonal abundance over a year's time during 1983 was only on the order of 2-8% for any of the _Gambusia_ populations.

At the trapping stations below the dam, a different pattern of seasonal abundances was observed (Table 1, Fig. 5). The G. _heterochir_ population increased markedly in abundance over the sampling period from less than 1% in 1953, 1956-57, 1968-69 and 1971, to approximately 70% in the January 1984 sample. In each year between 1980 and 1984, actual highest abundances were generally achieved in the summer, however, the greatest relative abundance of G. _heterochir_ occurred in the last (January 1984) collection. The proportion of hybrids has remained relatively stable throughout the sampling period subsequent to the repair of the dam, varying from about 5-20% of all _Gambusia_ collected in the traps located below the dam when the spring 1980 collection (N=3) was excluded. In terms of seasonal variation, the hybrids tended to reach highest levels during winter or spring collections, though much variation throughout each year was evident. However, compared to previous decades, the numbers of hybrids and G. heterochir in the area below the dam have increased greatly since the dam was repaired. Gambusia affinis abundances in the traps below the dam varied widely from over 80% to nearly 20% of all Gambusia trapped. In general, lowest G. affinis abundances were weakly correlated to warm water temperatures during summer months and highest abundances weakly correlated with winter-time collections. Fall collections were found to have both high and low relative abundances of this species.

At the seining station established in the stenothermal springs area below the dam (Station R), the proportion of G. heterochir rose from very low levels (0-7%) before the dam was repaired to approximately 45% of the Gambusia one year later (Table 1, Fig. 6). In the period from 1981-83, the proportion of G. heterochir continued to rise and at the end of the sampling period averaged approximately 80% of the Gambusia taken by seine. Hybrids also appeared in the seine samples (Fig. 7), undoubtedly as a result of the large increase in G. heterochir abundance in this downstream spring area. The proportion of the Gambusia population accounted for by the hybrids has varied between 2-13% with highest levels occurring in the winter collections of each year. Over the course of this study, hybrid abundance patterns appeared to be similar to those found at the trapping stations above the dam, both exhibiting decreasing abundance over time. Abundance of G. affinis at the seining station (Fig. 6) also have substantially decreased when compared to previous decades. The greatest abundance found during 1980-1984 was slightly less than 60% and their abundance percentage has declined to approximately 10% in the January 1984 sample. Prior to 1980 they had dominated the area being more than 90% of the poeciliids captured.

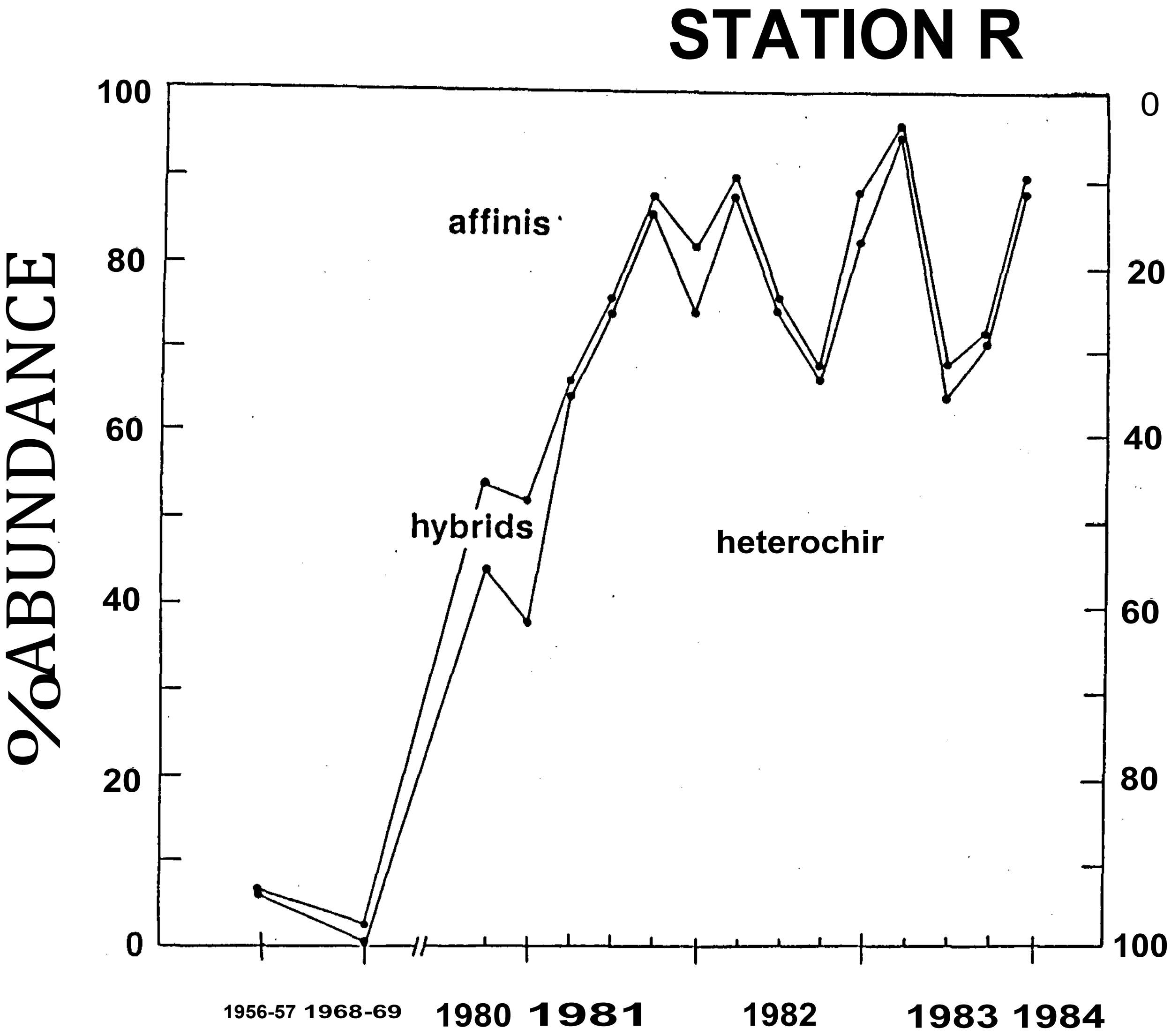

Figure 6. Relative abundances of Gambusia heterochir, G. affinis and hybrids taken by seine at downstream stenothermal springs, Station R. Data from 1956-57 (Hubbs 1959) and 1968-69 (Hubbs 1971) are shown for comparison.

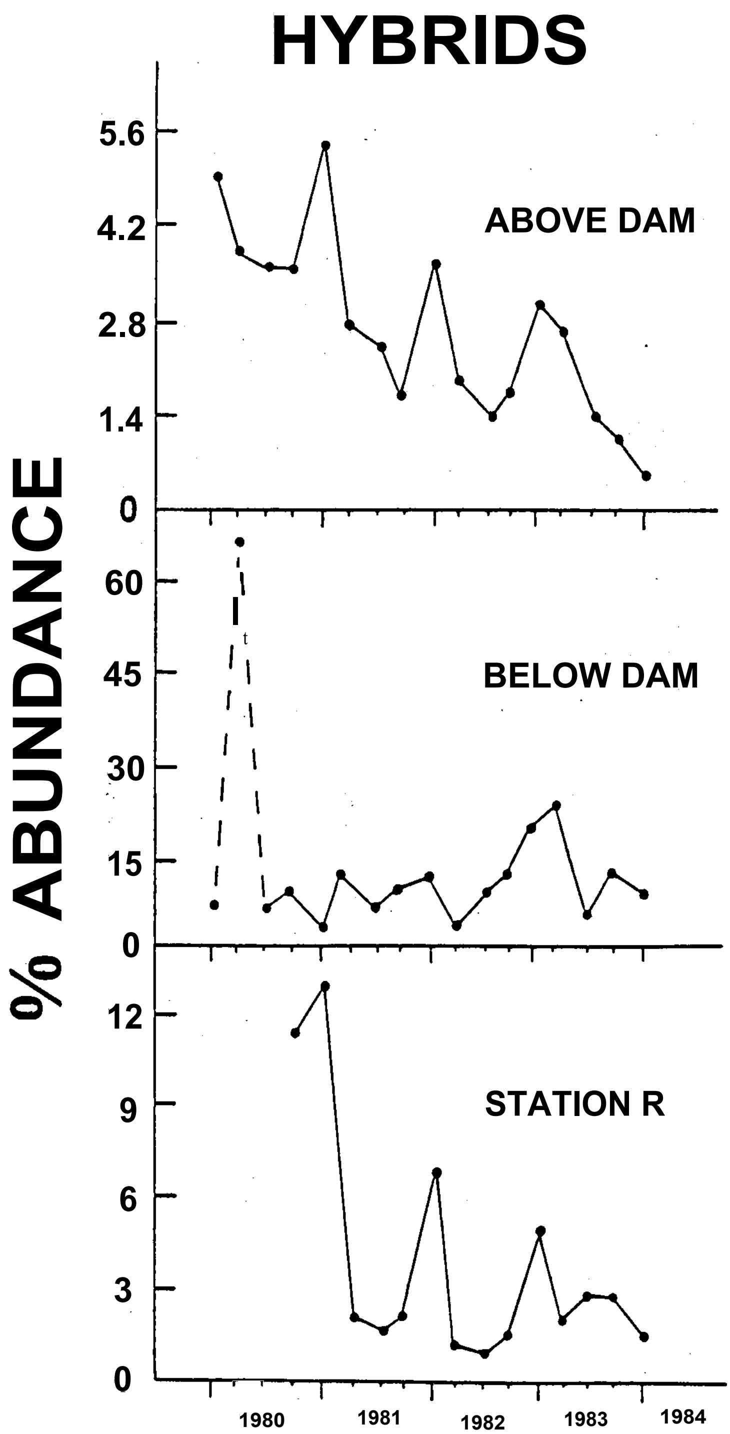

Figure 7. Changes in relative abundances of hybrid Gambusia taken at stations above the upper dam, below the dam and at Station R at Clear Creek during the 1980-84 sampling period. Percentages are reported as the percentage of all Gambusia that are represented by hybrid individuals in each environment in the Clear Creek collections.

The abundance of the introduced Lucania parva varied seasonally and predictably (Fig. 8). Highest levels were always found during the winters of each year following their discovery in Clear Creek in 1980. Lowest levels of Lucania always occurred during the spring or summer collections. The ease of capture of Lucania in the minnow traps and in seines and its distinctiveness indicate that this species was in all likelihood not present in Clear Creek during previous investigations. Numerous Lucania were collected in the 1980's by the same techniques and at the same locations that were sampled in the prior studies that yielded no Lucania.

Hybrid index scores comparing the Gambusia populations taken during this study with those reported by Hubbs (1959, 1971) are shown in Tables 2 and 3 for males and females, respectively. Few changes are evident in the overall range of index values despite the presence of the hybrid swarm for more than 30 years and 60 generations. In contrast to the 1956-57 and 1968-69 samples where few adult G. heterochir or hybrids were taken at stations below the dam, the 1980-83 data corroborate the observed increase in G. heterochir abundance in the area below the dam and at the stenothermal Station R. An apparent result of the increase in G. heterochir abundance has been an increase in the abundance of hybrids.

The proportion of hybrids in samples of juveniles at all stations was generally much lower than the proportion of hybrids in samples of adults (Table 4). In contrast, the proportion of juveniles to that of adults in the parental species were not significantly different overall based on the results of a nonparametric sign test (Sokal and Rohlf 1969). As Gambusia at Clear Creek usually have two generations per year, juveniles would enter the adult population in no more than two sample intervals. Conversely, juveniles are the

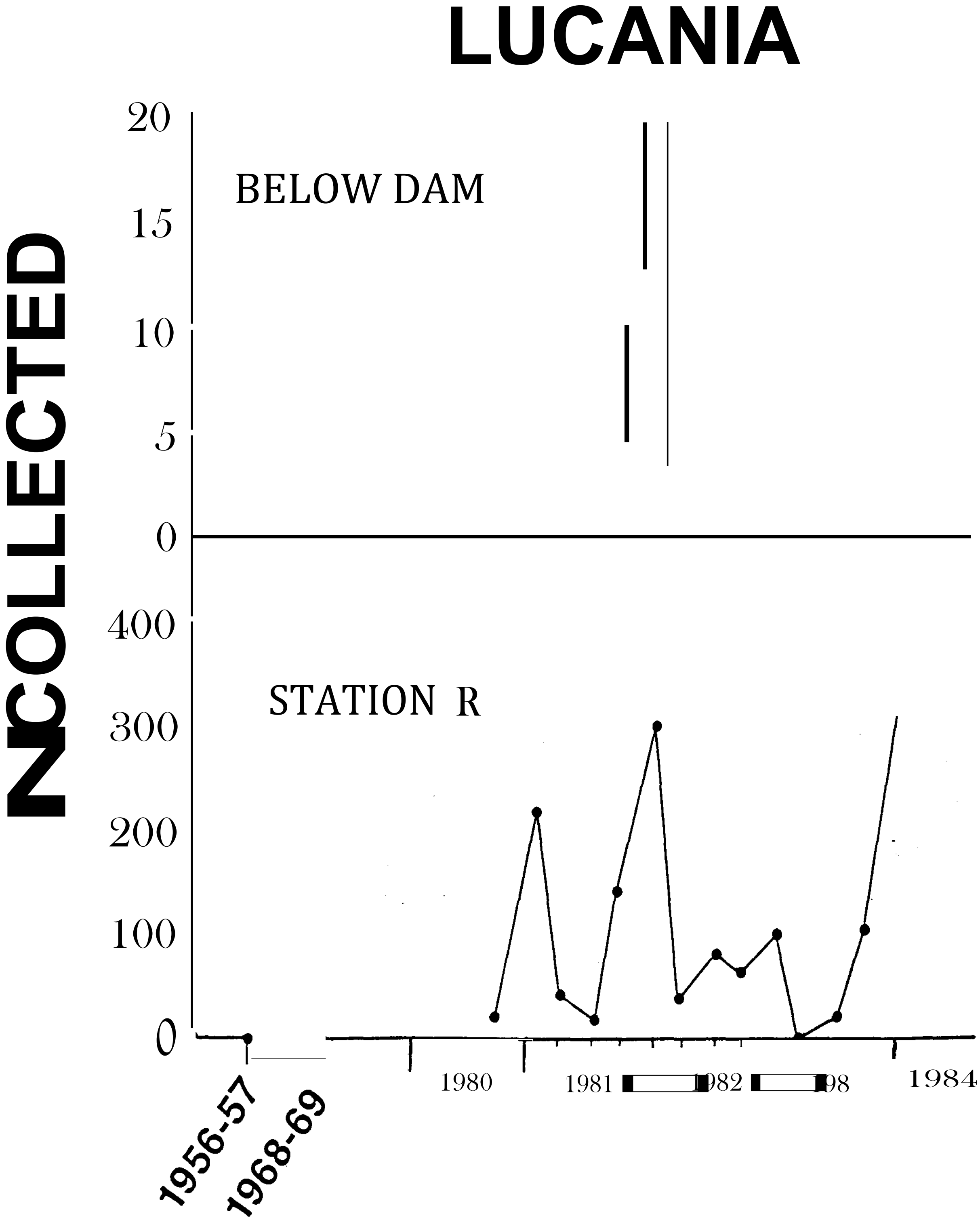

Figure 8. Numbers of Lucania parva taken in each season in Clear Creek collections below the upper dam and at Station R. No Lucania were taken above the upper dam. Lucania in Below Dam collections were taken in minnow traps, those at Station R were collected with seines.

Table 2. Percent frequency distributions of hybrid index values for adult male Gambusia at three Clear Creek sampling stations for three time periods. Data from 1956-57 and 1968-69 from Hubbs (1959, 1971).

	Hybrid Index Value	ABOVE DAM 1956-1957	ABOVE DAM 1968-1969	ABOVE DAM 1980-1983	BELOW DAM 1956-1957	BELOW DAM 1968-1969	BELOW DAM 1980-1983	STATION R 1956-1957	STATION R 1968-1969	STATION R 1980-1983
affinis	46			0.1						0.3
	47					0.1	0.7			
	48					0.3				0.3
	49			0.2		1.0	1.4		0.6	0.6
	50		0.2	1.1	2.3	2.0	2.1	0.1	1.3	0.9
	51		0.3	0.9	5.0	5.3	2.8	1.0	4.0	3.4
	52	1.0	0.8	2.0	9.0	11.0	7.6	4.0	7.5	6.0
	53	1.0	1.0	3.0	14.5	19.7	4.8	7.0	12.5	8.6
	54	2.0	1.5	3.2	20.0	17.5	8.3	16.0	18.0	6.0
	55	4.2	1.8	3.5	21.3	15.5	9.7	24.5	15.7	3.7
	56	8.2	2.0	2.3	14.2	11.0	1.4	18.5	10.7	1.7
	57	6.2	1.0	1.3	8.7	6.2	3.4	12.0	11.9	1.7
	58	3.0	1.0	0.7	1.9	4.0		6.3	6.8	0.6
back-crosses	59	2.5	0.7	0.7	1.2	1.7	1.4	2.0	3.0	0.3
	60	2.0	0.7		0.3	1.0	2.8	1.0	1.3	
	61	1.0	0.7	0.1	0.3	0.5	0.7	0.2	1.0	
	62	1.0	0.6	0.4		0.1	1.4	0.1	0.3	
	63	2.0	0.5			0.4	0.7	0.1	1.0	
	64	0.6	0.6	0.2		0.3	0.7	0.1	0.2	
	65	1.0	0.3	0.1		0.1		0.4	0.1	0.3
hybrids	66	1.0	0.4	0.1		0.1	0.7		1.0	0.3
	67	0.6	0.7	0.2		0.2	0.7		0.3	0.3
	68	1.0	0.7	0.2		0.2		0.3	0.3	
	69	3.0	0.7	0.5		0.4	4.1	0.5	0.4	0.3
	70	2.5	0.7	0.4		0.4	1.4	1.2	0.4	0.9
	71	2.0	1.0	0.8	0.7	0.1	2.1	1.0	0.6	1.7
	72	2.0	0.8	0.4		0.3	3.4	1.8	0.5	1.4
	73	1.8	0.7	1.1		0.2	2.8	1.0	0.4	1.7
	74	1.7	0.9	0.6		0.1	4.1	0.8		1.7
	75	1.0	0.9	0.2		0.1	3.4	0.7	0.3	1.7
back-crosses	76	1.0	0.5	0.1		0.1	0.7		0.6	0.6
	77	1.2	0.4	0.1			1.4		0.5	0.6
	78		0.4	0.1		0.1			0.3	
	79	0.4	0.3	0.2		0.1				
	80	0.4	0.4			0.1				0.6
	81	0.4	0.6	0.1					0.3	0.6
	82		0.7							
heterochir	83	0.4	1.0							
	84		0.8							0.3
	85		1.7	0.1						
	86		2.5	0.1						
	87	0.4	4.8	0.4						
	88	0.4	6.0	0.1			0.7			0.3
	89	0.8	8.3	1.4			2.8			0.3
	90	0.6	9.0	1.5			2.8			2.9
	91	2.0	10.5	4.1			2.1	0.4		2.6
	92	3.4	10.0	5.3			4.1	0.5		3.7
	93	5.8	10.0	8.5			0.7	0.6		6.6
	94	8.7	6.6	11.2			4.1	0.4		6.9
	95	6.8	3.6	9.0			2.1	0.4		6.3
	96	5.5	2.0	10.8			1.4			7.7
	97	1.8	1.0	8.8			1.4			7.1
	98	0.8	0.2	6.3			2.1			4.6
	99		0.4	3.0			0.7			2.0
	100			2.9			0.7			1.1
	101			1.1						0.9
	102			0.1						0.3
	103			0.1						
		401	2518	854	334	1648	145	929	868	350

Table 3. Percent frequency distributions of hybrid index values for adult female **Gambusia** at three Clear Creek sampling stations for three time periods. Data from 1956-57 and 1968-69 from **Hubbs** (1959, 1971).

		ABOVE DAM			BELOW DAM			**STATION** R		
	Hybrid Index Value	1956-1957	1968-1969	1980-1983	1956-1957	1968-1969	1980-1983	1956-1957	1968-1969	1980-1983
	69						0.4			
	70			0.1						
	71			0.3		0.2	1.4		0.2	
	72			0.3	0.3	0.2	0.7	0.1	0.4	0.6
	73	0.5	0.3	0.8	2.1	0.8	2.9	0.4	1.0	
affinis	74	0.7	0.5	0.9	5.0	2.2	5.4	2.0	2.6	1.9
	75	0.7	0.5	1.5	6.2	5.3	9.3	4.2	6.2	
	76	4.2	0.9	2.4	14.2	12.0	7.9	11.5	11.8	5.1
	77	6.5	1.0	3.1	15.4	17.6	14.0	18.2	18.9	8.9
	78	9.2	1.5	4.1	20.8	20.9	5.7	20.5	21.2	5.1
	79	8.2	1.9	2.6	16.0	18.0	7.5	19.0	18.0	7.6
	80	9.6	1.4	2.8	12.0	11.6	3.9	12.0	11.0	5.7
	81	6.5	1.2	3.2	4.5	6.8	7.2	6.2	5.0	5.7
back-	82	1.4	1.0	2.7	2.1	2.6	2.5	3.0	1.8	3.8
crosses	83	2.0	1.0	1.0	0.7	1.3	3.6	1.0	1.1	1.3
	84	1.7	0.6	1.5		0.3	1.4	0.3	0.3	0.6
	85	1.2	1.0	1.1		0.1	1.1	0.1	0.1	1.3
	16	1.2	0.7	0.3		0.1	1.8		0.1	0.6
	87	1.8	0.6	0.3			1.1		0.1	
hybrids	88	0.8	0.6	0.6	0.4		0.7			0.6
	89	1.2	0.7	0.5					0.1	0.6
	90	0.8	0.7	0.6			0.4			0.6
	91	0.8	0.8	0.2						0.6
back-	92	0.8	0.6							0.6
crosses	93	1.2	1.0	0.5			0.4			
	94	0.9	1.5	0.7						0.6
	95	0.3	1.6	1.2			1.1			
	96	1.8	3.6	1.8			2.9	0.1		
	97	2.0	5.4	2.8			1.1	0.4	0.2	0.6
	98	5.0	7.3	4.1			2.2		0.1	1.9
	99	7.0	9.0	5.9			3.2	0.3		3.2
	100	6.3	12.3	9.1			1.8	0.5	0.1	3.8
	101	4.9	12.2	8.6			2.2			3.8
heterochir	**102**	3.5	11.6	10.3			1.1			6.3
	103	3.0	9.0	8.6			1.8			7.6
	104	1.0	6.8	6.6			1.1			5.7
	105	0.5	2.3	3.5			1.4			7.0
	106		0.8	3.1			0.7			5.7
	107		0.2	1.5						2.5
	108		0.1	0.5			0.4			
	109		0.1	0.2						
	110			0.2						
		281	4522	1296	319	11,372	279	1274	3196	158

Table 4. Percentage of the collections in which adults were proportionately more abundant than juveniles for Gambusia heterochir, G. affinis and hybrids at stations above the upper dam, below this dam and at Station R during the current study. Asterisks refer to chance probabilities of obtaining equal proportions of adults and juveniles of each species or hybrid in a sample interval based on the results of a nonparametric sign test. Values with no asterisk are not significantly different from equal proportions of adults to juveniles.

	G. heterochir	hybrids	G. affinis
ABOVE DAM	58%	76% *	29%
BELOW DAM	44% (1 tie)	100% ***	29%
STATION R	27%	93% **	71%
TOTAL	44.8% (P=0.38)	89.6% ***	41.7% (P=0.47)

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

product of the adults in the prior sample (or two) and become part of the adult sample of the following date (or two). Nevertheless, juvenile hybrid frequency generally is lower than that of the preceeding or following adult samples. Two factors may be involved, biological or methodological. The latter is unlikely as adults were allocated to species or as hybrids by the same procedures as were juveniles (visual inspection) after which the adults were allocated following the coding procedures with a very high concordance (greater than 99%). Therefore, it is probable that the difference has a biological basis. The low proportion of hybrid juveniles contrasted with their parents is in accord with some level of mating preference or perhaps an early developmental mortality of hybrids. The increase in hybrid proportions evidenced by adults suggests heterotic phenomena, in particular with F1 hybrids as adult hybrid males, especially, consistently have more individuals with an intermediate index value (69-75) than for those codes expected to be most representative of backcross individuals (65-68 and 77-82, respectively, Table 2). This pattern is, however, less clear with the data from adult females (Table 3).

Discussion

The Clear Creek hybrid swarm has been in existence for over 30 years and, yet, genetic fusion of the parental species has not occurred. The hybrid index samples show that G. heterochir and G. affinis are no more similar morphologically to each other now than they were during the 1956-57 or the 1968-69 studies or even the 1953 sample. The presence of fewer presumed backcross hybrids than F1 hybrids suggests that backcrossed individuals may be at a selective disadvantage in Clear Creek. Hubbs (1971) concluded that there may be insufficient intermediate ecological niche space for the successful establishment of these backcrossed individuals and this limitation may provide a sufficient reproductive isolating mechanism to prevent the genetic introgression of these Gambusia. Our results are in agreement with this hypothesis.

Clear Creek has been extensively modified. Presumably, Clear Creek originally was a clear springrun which flowed from the uppermost spring pool, which was likely slightly smaller than the present pool above the dam, to its confluence with the San Saba River, approximately 5 km downstream. Most, if not all, of the springrun was undoubtedly inhabited by the endemic spring organisms, including G. heterochir, Hyalella texana and Ceratophyllum sp. Human activities near the spring pool have been of long duration. Abundant indian artifacts on the top of the cliff surrounding the headsprings are indicative of a settlement in the area and also attest to the long stability of the spring flow. Spanish missionaries and settlers colonized the surrounding area of the San Saba River basin during the 17th century (San Saba Presidio and Mission were 15 and 20 km east of the study area); however, their presence or activities at the Clear Creek headsprings remains uncertain.

By the 1880's, the present upper dam had been built. The actual date of its construction is unknown but its purpose was related to irrigation as very old and abandoned irrigation ditches are still evident from the ends of the dam to large open flats about 1 km downstream and the Wilkinson family members recall their use for irrigation. During the late 1920's, this dam was repaired and cement facings joining portions of the limestone walls were made. During the 1930's, a second dam was constructed approximately 1.5 km below the upper dam which caused stream waters to back up to the base of the first dam and created an extensive quiet water pool below this dam. Shortly after the second dam was built, a third dam was constructed approximately 1.5 km below the second. The pool elevation from this third dam equalled that of the pool formed by the second dam, thus creating a single 3 km pool below the upper dam with a nonfunctional earth dam/dike in the middle of its length. This extensive pool created a great amount of eurythermal, shallow water habitat which undoubtedly allowed the existing G. affinis population to flourish throughout Clear Creek as far upstream as the upper dam. Although it is uncertain exactly how far downstream in Clear Creek G. heterochir originally extended or whether or not a hybrid zone existed, the endemic biota probably extended almost to the San Saba River confluence and the hybrid zone, if present, was probably downstream of the present upper dam. In addition, without the dams, the difference between stenothermal and eurythermal environments within Clear Creek was probably much less abrupt and more distinctly separated than now occurs directly above and below the upper dam. These modifications to Clear Creek appear to have caused the original endemic biota to become essentially restricted to the area above the upper dam. A fourth dam has also been built on Clear Creek about 0.4 km below the third dam which has further impounded and slowed the Clear Creek stream waters. Because the second and third dams had already created the environmental conditions which led to the elimination of the endemic biota below the headsprings, this fourth dam has not contributed directly to the decrease in their abundances below the headsprings.

Poplars were planted on the upper dam during the 1930's to provide shade for fishermen. Strong winds have caused several of these to uproot causing the containment wall of the dam to be weakened and in places, to break. During the latter 1930's, the dam was again repaired and the pool level was raised about 0.5 m (to near the dam's crest). The increased water pressure augmented the damage from the toppled trees causing several breaches in the dam. Nutria were introduced into the San Saba River during the 1940's, entered Clear Creek and they began tunnelling into the pond banks and earth dam resulting in the ultimate state of disrepair which necessitated the dam's renovation in 1979.

The pattern of Gambusia relative abundance at the trapping stations above the dam indicates that the dam rebuilding activities disrupted the Gambusia in the area immediately above the dam. The observed abundances suggest that following the construction activities, Gambusia populations near the dam were dispersed and then recolonized the trap area by the winter of 1980-81 as the disturbed vegetation reestablished above the renovated dam. Over the course of this study, the G. affinis population above the dam became less abundant indicating that Gambusia heterochir appears to have a distinct survival advantage in the stenothermal environment of the upper pool.

Since the **dam** was rebuilt, G. affinis immigrants from the downstream population have been unable to gain access to the upper pool environment. This factor was considered by Hubbs (1971) to be most important in the continued presence of hybrids in the pool above the dam. The data in the present study suggest that G. affinis are not persisting in the upper spring pool and if trends continue, may be eliminated from this portion of Clear Creek in only a few more years. Without the presence of G. affinis, hybridization above the dam may not be maintained, thus eliminating the hybrid swarm at the headsprings of Clear Creek.

Below the dam and at Station R, we had originally predicted that the Gambusia populations would reestablish their former levels of abundance (i.e. G. affinis with few or no G. heterochir or hybrids), since few G. heterochir had ever been found below the upper dam, even in the limited stenothermal environment at Station R. This circumstance, described in all previous studies, suggested low survival potential of G. heterochir in the presence of abundant G. affinis populations. The data in our present study, however, fail to support this expectation.

We suspect that large numbers of Gambusia heterochir likely entered the lower pool when the water level in the upper pool was lowered during the dam reconstruction. However this transfer is insufficient to explain the continued presence four years later of this species below the upper dam. Our data show consistent increases in the abundance of G. heterochir during this study in the areas immediately below the dam and also at Station R. To account for these changes, several factors may be involved. The physical environments below the dam and at Station R may be different now compared to those encountered in previous decades; the released G. heterochir may have genetically swamped the downstream G. affinis populations; or there may have been a change in the biotic interactions of the species inhabiting the creek below the upper dam. This change in biotic interactions could result in the observed high abundance of G. heterochir by either favoring G. heterochir directly or by adversely affecting G. affinis, thus favoring G. heterochir indirectly.

The first possibility seems unlikely as the physical and chemical conditions downstream have not appreciably changed since the 1950's. There is a slightly more organized (channeled) spring seep along the downstream eastern end of the dam which was created to relieve erosion of the eastern part of the dam during the dam reconstruction and there may also be a very slight increase in downstream spring head pressure at the spring seeps at Station R since the dam no longer leaks as much water. Neither of these slight differences is enough to explain the magnitude of the increase in G. heterochir's abundance nor its persistence during this study at either the trapping stations below the dam or at Station R. The second factor may be involved but this also seems unlikely as the two species have maintained their genetic integrities throughout over 30 years of study without noticeable change.

Shortly after the **dam** was renovated, the rainwater killifish, Lucania **parva**, was noted in Clear Creek along the eastern shore below the upper dam. This coastal cyprinodontid is not native to the Edwards Plateau including Clear Creek (Hubbs 1982), but is found throughout much of the Pecos River basin to the west of Clear Creek as well as coastal areas to the east. The source of the Clear Creek stocks of Lucania are unknown; however, it seems likely that they were first released into Clear Creek not long before 1980, presumably either as or with leftover bait. The consistent increases in their numbers that we found in this study suggest that Lucania were introduced near the time of the **dam** reconstruction and have not yet reached their carrying capacity in Clear Creek.

Lucania and G. affinis are similar **in** many aspects of their ecologies, especially with respect to their food habits, habitat preferences and tendencies to migrate seasonally in order to exploit the warmest available environments. At Leon Creek (Pecos Co., Texas) where Lucania, G. affinis and the spring adapted G. nobilis are found, Lucania and G. affinis inhabited similar habitats (eurythermal environments) in contrast to the stenothermal habitat preferences of G. nobilis (Hubbs et al. 1978). In addition both Lucania and G. affinis had many fewer **amphipods** in their diets than did the spring adapted G. nobilis, especially in habitats with significant spring seeps present. Finally in Leon Creek, although each of these species were found to be relatively generalized in the feeding ecologies, Lucania and G. affinis were much more similar to each other in their resource use than were either to G. nobilis. The ecological situation found at Leon Creek is strikingly similar to that found at Clear Creek. Lucania and the eurythermal adapted G. affinis share extensive niche overlap and both differ from the syntopic stenothermal adapted **Gambusia** (G.

nobilis or G. heterochir in Leon Creek and Clear Creek respectively). It is therefore probable that the observed increase in Lucania abundance that we have found in this study is at the expense of G. affinis abundance rather than G. heterochir. The correlation between the relative abundances (arcsine transformed) of G. affinis and Lucania was found to be $r=-0.43$ ($P=0.06$), further suggesting that the introduction of Lucania may well be the difference in biotic interactions responsible for the increased abundance of G. heterochir below the upper dam.

As Lucania is an oviparous cyprinodontid, hybridization with the viviparous poeciliid, G. heterochir is precluded and thus genetic contamination from this species is not possible. In contrast to the outcome of many species introductions, this is apparently an instance where the release of an exotic has had a beneficial impact on an endangered species in its natural habitat.

It is apparent that the alteration of upstream immigration patterns of the Gambusia at Clear Creek due to the repair of the breaks in the upper dam has caused a significant decrease in the extent of the long-standing hybrid swarm. Since the reduction in hybrid abundance occurred much more rapidly than the decrease in G. affinis abundance at the stations above the dam, it seems likely that naive immigrants have indeed played a significant role in the production of hybrids as was first suggested by Hubbs (1971). Our confirmation of this finding may be of great importance in situations where sexually naive (juvenile) fishes are stocked in the range of possible hybridizing species. In these situations, the use of such naive individuals may exacerbate problems associated with genetic contamination of the species' gene pools.

Finally, we note that although G. heterochir appears relatively secure in its pool above the dam following the dam reconstruction, we recognize that the Clear Creek headsprings are an extremely limited habitat and that efforts to return this endangered species to its former levels of abundance would necessitate the ultimate removal of the dams **which** have allowed the large populations of G. affinis and L. parva to become established.

Acknowledgments

This study has received the full cooperation of the Office of Endangered Species (U.S. Fish and Wildlife Service), the Texas Parks and Wildlife Department and especially the Wilkinson family, who until recently owned the land surrounding the Clear Creek headsprings and who have demonstrated a strong commitment and a commendable level of environmental concern for the preservation and conservation of the Clear Creek gambusia and the Clear Creek environment.

We also acknowledge the efforts of S. M. Dean, A. A. Echelle, A. F. Echelle, G. P. Garrett, L. Garrett, D. S. Marsh, E. Marsh, D. Mosier, J. Bentley, F. Potter and W. McPherson in rebuilding the dam as volunteers.

Numerous University of Texas students, spouses and friends assisted with the field work and we thank them for their help.

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