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Author(s): Stephen C. Stearns and Richard D. Sage
Source: *Evolution*, Vol. 34, No. 1 (Jan., 1980), pp. 65-75
Published by: Society for the Study of Evolution
Stable URL: http://www.jstor.org/stable/2408315

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Evolution, 34(1), 1980, pp. 65–75

MALADAPTATION IN A MARGINAL POPULATION OF THE MOSQUITO FISH, *GAMBUSIA AFFINIS*

STEPHEN C. STEARNS[1] AND RICHARD D. SAGE
Department of Zoology, University of California and
Museum of Vertebrate Zoology, University of California, Berkeley, California 94720

Received July 3, 1978. Revised June 14, 1979

Field biologists commonly assume that the organisms with which they deal are well adapted—even optimally adapted—to local circumstances. Both Ehrlich and Raven (1969) and Endler (1977) have de-emphasized the role of gene flow in preventing large scale geographic differentiation and local adaptation. This paper documents a case in which gene flow may have prevented small scale local adaptation in one population of mosquito fish, *Gambusia affinis*. It carries two messages: field-workers should check the assumption that their study organisms are adapted to the local environment because that assumption does not always hold, and there are limiting cases involving high dispersal rates over short distances in which gene flow can overwhelm local selection pressures.

Evidence that local populations are being maintained away from the locally optimum phenotype is rare. Camin and Ehrlich (1958) analyzed the banding patterns of water snakes (*Natrix sipedon*) on islands in Lake Erie. They showed that on the islands, where snakes were probably confined to a band of sandy substrate near shore by lack of water inland, patternless, light-colored snakes were most common. In contrast, on the mainland where water snakes inhabit wooded streams with darker, more complex substrates, richly patterned, dark-colored snakes predominated. However, a small proportion of the island snakes were patterned, and therefore maladapted to the local environment, an observation that Camin and Ehrlich explained by invoking gene flow from the mainland.

To understand why we did the experiments reported here, the reader must realize that at the outset we did not conceive this study as a problem in population structure, gene flow, and maladaptation. We thought we were working with microgeographical variation in life-history traits that could be explained either by local adaptation or by developmental sensitivity to early environment. Let us begin with the field-data that demanded an explanation.

In April, 1975, and again in April, 1976, we sampled two populations of mosquito fish at Armand Bayou, Texas, a nature reserve about 50 km southeast of Houston. The sampling date was chosen to yield the first brood of the year. One population we seined along the margins of the brackish (10‰) estuary that forms the bayou; the other we seined from a series of pools 150–300 m up a small, freshwater (0‰) steam that drained into the bayou through an intermittently dry connection. The course of the stream had been modified by road construction in 1967 and 1969 (pers. comm., N. Gamos, Trans. Dept., Harris Co., Texas). Since that time, 12–24 generations elapsed prior to the collection of the first field sample, and 14–27 generations prior to the second (using 2–3 generations/year).

In both 1975 and 1976, females at a given size (75 mg dry weight) from fresh water had fewer (16 vs. 25 in 1975, 13 vs. 20 in 1976) and larger (1.34 vs. 1.16 mg in 1975, 1.20 vs. 1.03 mg in 1976) young than females from brackish water, and they had smaller ratios of dry weight of brood to dry weight of mother (0.27 vs.

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[1] Current address: Reed College, Portland, Oregon 97202.

TABLE 1. *Summary of field data. Comparisons of fresh and brackish population of* Gambusia affinis *at Armand Bayou, Texas, in April, 1975 and 1976. Confidence limits (±2 SE) for traits at given weights are estimated from regression equations.* P-*values for fresh-brackish comparisons result from analysis of variance or covariance* F-*ratios.*

	1975			1976		
Traits	Fresh	Brackish	*P*	Fresh	Brackish	*P*
Sample size:						
Adult females	144	68		206	71	
Adult males	46	13		71	28	
Number of young in 75 mg females	16.0 ± .3	25.4 ± 1.0	.001	13.3 ± .3	19.6 ± .3	.001
Weight (mg) of early-eyed eggs	1.34 ± .04	1.16 ± .04	.001	1.20 ± .06	1.03 ± .06	.001
	(n = 68)	(n = 36)		(n = 110)	(n = 37)	
Dry wt of brood/dry wt of female, for 75 mg females	0.27 ± .04	0.31 ± .09	.001	0.22 ± .04	0.25 ± .09	.001
Length (mm SL) of 75 mg females	28.2 ± .14	28.5 ± .28	.001	27.8 ± .15	.28 ± .24	.104
Length (mm SL) of 30 mg males	20.8 ± .24	21.3 ± .41	.023	21.2 ± .20	21.4 ± .27	.164

0.31 in 1975, 0.22 vs. 0.25 in 1976) (Table 1). All these differences were highly significant ($P < .001$). Differences in the condition (fatness or thinness) of males and females from fresh and brackish water were significant in 1975, not significant in 1976, and small in both cases. (Condition is expressed as the regression of length on the natural logarithm of weight; the significance of differences in condition, as for size of brood and other weight-dependent traits, was calculated by first estimating the effects of weight and removing them from the data, then performing an analysis of variance on the residuals.)

The data fit at least three hypotheses which were not mutually exclusive: (1) the fish were getting more to eat in brackish water, thus were able to put more energy into reproduction without much sacrifice in condition, and there were no major genetic or developmental differences between the stocks; (2) each population was distinct and adapted to its local environment; the differences between them had a genetic basis, and investigation of what caused the differences should shed light on the evolution of life-history traits; (3) the two populations were genetically identical, but the fish had a developmental sensitivity to salinity that resulted in the production of different phenotypes in each environment.

With these hypotheses in mind, we designed a set of experiments to assess the relative importance of hypotheses (2) and (3). We felt that hypothesis (1) was probably always a factor of some importance in the field but was probably not a primary factor in this case because of the small differences in condition of the fish; we eliminated it from the experiments by controlling food level.

BACKGROUND

The organism.—Gambusia affinis is a small, sexually dimorphic, poeciliid fish native to the Gulf Coast, lower Mississippi drainage, and Atlantic lowlands of the United States and Mexico (Rosen and Bailey, 1963). Since 1905 it has been spread around the world for mosquito control, and now inhabits freshwater and brackish areas in Europe, Asia, Africa, South America, and Oceania (Seale, 1905; Krumholtz, 1948). Both genetic and social factors have been implicated in the control of age and size at maturity of male poeciliids, which stop growing when they mature (Borowsky, 1973; Kallman and Schreibman, 1973; Kallman, 1976; Sohn, 1977*a*, 1977*b*). Females have indeterminate growth and are the heterogametic sex (Roberts, 1965; Chen and Ebeling, 1968). Fertilization is internal, accomplished after a relatively simple courtship (Rosen and Tucker, 1961; Carlon, 1969; Peden, 1972, 1973; Martin, 1975), and young are born alive after a gestation period of 16 to 80 days, depending on temperature, food supply, season, and local adaptation (Hildebrand, 1917; Hubbs, 1971; Stearns, 1975). Females can retain viable sperm for several months, and at times carry two broods at different stages of development (Scrimshaw, 1944; Hubbs, 1971; Stearns, 1975). Most populations have broad salinity (Renfro, 1959; Ahuja, 1964) and thermal (Otto, 1973, 1974; Johnson, 1976) tolerances.

METHODS AND EXPERIMENTAL DESIGN

The stocks for all experiments were captured in the field on April 27, 1976 with a two-man seine (15 m long, 6.5 mm mesh), shipped to Berkeley, and maintained in the type of water (fresh or brackish) in which they were captured. Over 20 females and 20 males were taken from each site. The stock tanks were 95 liter aquaria with under-gravel filters; fish were fed Tetramin flakes once per day.

Experiment I.—In Experiment I, we used the progeny of 18 females captured in fresh water and 31 females captured in brackish water in the field at Armand Bayou. We made up fresh (0.5‰) and brackish (10.0‰) media with Berkeley city water and Instant Ocean salts (trace elements included). When a female gave birth, we put half her brood into fresh water and the other half into brackish water. After one week, we again split the broods, putting half into fresh and half into brackish water. We then combined the broods from different females to get 20 fish per replicate (a 19 liter aquarium). In any one replicate, the fish varied in age over a four day range. Thus there were eight treatments according to origin of parents and the salinity of the water in which the first week of life and the rest of life were spent. These are denoted FFF, FFB, FBF, etc. with the number of replicates given in Table 2. Each aquarium received 15 mg of ground Tetramin flakes per day for the first week, then 20, 25, 40, 50, 60, . . . 130 mg per day in successive weeks. After the 13th week, each aquarium received a constant 6 mg per fish per day. All aquaria were maintained at 25 ± 1 C, and every aquarium or jar was emptied, cleaned, and refilled with fresh water once a week. When a given replicate was 86 days old, the fish were anaesthetized in soda water (CO_2 is the effective ingredient), then weighed (±0.1 mg) and measured (±0.5 mm). Handling mortality was <1%. We maintained each replicate until every surviving female had given birth at least once.

Experiment II.—Experiment II differed from Experiment I in five respects. First, the fish were born of females that had spent their whole life in the laboratory; we used the progeny of eight females from the FBB and six females from the BBB treatments of Experiment I. Second, we did not give the fish different treatments in the first week of life; from the day of birth we reared half of each brood in fresh and half in brackish water. Third, we raised the fish in isolation in 0.95 l jars, to minimize the effects of social interactions on growth rates. Fourth, we used strictly defined media, not Berkeley city water: brackish = 114 liter distilled water + 1.3 kg Instant Ocean salts + 15 ml Instant Ocean trace elements + 8 g $NaHPO_3$ + 10 g $NaHCO_3$; fresh = 114 liter distilled water + 65 g Instant Ocean salts + 1 ml Instant Ocean trace elements + 7 g $NaHPO_3$ + 10 g $NaHCO_3$. Both media had pH = 6.8–6.9. Fifth, we did not weigh the food for each fish, but fed them slightly more Tetramin than they could eat once each day. Water was changed completely once a week, and the fish were weighed and measured when 86 days old, then reared to morphological maturity (complete development of gonopodium in males, appearance of cloacal spot in females). The four treatments in Experiment II (FF, FB, BF, and BB) were defined by the origin of the grandparents and the medium in which the fish were raised. Eight of the fish in the FF treatment were given heavy medication (250 mg tetracycline hydrochloride, 60 mg nitrofurazone, 25 mg furazolidone, 2 mg methylene blue, and 0.04 mg sulfadimidine per 40 l of me-

TABLE 2. *Length, weight, and survival of fish 86 days old. Experiment I. All replicates started with 20 fish.*

Treatment conditions experienced			Number of replicates	Number of fish	Length (mm) ± 2 SE	Weight (mg) ± 2 SE	Survival mean ± 2 SE
Mother	Week 1	Rest of life					
Fresh	Fresh	Fresh	2	26	15.2 ± 1.0	76.3 ± 16.5	13.0 ± 6.0
Fresh	Fresh	Brackish	4	75	17.3 ± 0.3	119.2 ± 6.2	18.8 ± 1.0
Fresh	Brackish	Fresh	3	30	15.4 ± 0.9	77.9 ± 14.1	10.0 ± 3.5
Fresh	Brackish	Brackish	4	79	17.1 ± 0.2	117.3 ± 4.5	19.8 ± 0.5
Brackish	Fresh	Fresh	1	9	16.8 ± 2.3	94.6 ± 34.8	9.0
Brackish	Fresh	Fresh	2	40	16.8 ± 0.1	104.1 ± 6.7	19.0 ± 0.0
Brackish	Brackish	Fresh	3	35	13.9 ± 1.0	59.4 ± 13.4	9.3 ± 2.9
Brackish	Brackish	Brackish	3	51	17.7 ± 0.5	129.1 ± 10.0	17.0 ± 3.0

dium) to test the hypothesis that they were suffering from a salinity-sensitive disease expressed only in fresh water.

Experiment III.—Experiment III differed from Experiment II in only two respects. First, the fish used were the progeny of fish caught in the field, as in Experiment I. Second, the stock came from Twin Reservoir in Hawaii, a freshwater reservoir at 670 m elevation that has had a resident population of *Gambusia* for about 70 years (ca. 140–210 generations). The Hawaiian *Gambusia* were introduced from Seabrook, Texas in 1905 (Seale, 1905), but whether they were taken originally from fresh or from brackish water at Seabrook is not known. The two treatments in Experiment III (FF or FB) were defined only by the medium in which the fish were raised (all fish having been born of females from fresh water).

RESULTS AND DISCUSSION

Experiment I.—Experiment I was designed to differentiate between the microgeographic adaptation hypothesis and the developmental sensitivity hypothesis. If the fish were adapted to their local environments, then most of the variability in Experiment I should have been expressed at the highest level—mother's origin—a result indistinguishable from a pattern caused strictly by maternal effects. If the fish were all genetically equivalent with respect to growth and reproduction, but were developmentally sensitive to their early environment, then most of the variability in Experiment I should have been expressed at the second level—environment in which the first week of life was spent.

In fact, the dominant effects on survival and growth were all at the third level—environment in which the rest of life was spent. At 86 days of age, regardless of mother's origin or early environment, there was a mean of 10.3 fish per replicate surviving in fresh water (51.5% survival) and 18.7 fish per replicate surviving in brackish water (93.5% survival, Table 2). Replicates of the same treatment differed significantly in survival, but no other differences among treatments were significant. Fish reared for the rest of their life in fresh water were shorter (15.0 vs. 17.3 mm SL, $P < .10$) and lighter (73.2 vs. 118.6 mg wet weight, $P < .05$) than fish reared for the rest of their life in brackish water (Table 2). The environment later in life and the replicate both had significant effects on the length and weight of the fish at 86 days of age.

The results of Experiment I fit neither the microgeographic adaptation hypothesis nor the developmental sensitivity hypothesis. We immediately suspected the laboratory fresh water supply, which was based on Berkeley city water. Therefore, we performed Experiment II using defined media made up from distilled water, to test for a bad water supply, and with a subset of the fish reared in fresh water on heavy medication, to check for a salinity-sensitive disease.

Experiment II.—In Experiment II, the survival results paralleled those of Experiment I: overall, fish reared in fresh water did not survive as well to 86 days of age

TABLE 3. *Survival to 86 days. Experiment II.*

Conditions experienced by		Number at 86 Days			
Previous generations	Self	Alive	Dead	Total	% Surviving
Fresh	Fresh	7	16	23	30.4
Fresh	Brackish	19	5	24	79.2
Total		26	21	47	$G = 9.74$, $P < .005$
Brackish	Fresh	13	7	20	65.0
Brackish	Brackish	16	4	20	80.0
Total		29	11	40	$G = 0.50$, $P > .5$
Combined	Fresh	20	23	43	46.5
Combined	Brackish	35	9	44	79.5
Total		55	32	87	$b = 10.47$, $P < .005$

(46.5% vs. 79.5%) as fish reared in brackish water (Table 3, $P < .005$). However, the result was sensitive to the conditions encountered by previous generations; fish whose grandparents came from fresh water showed a large and significant difference in survival (30.4% in fresh vs. 79.2% in brackish), whereas those whose grandparents came from brackish water showed a smaller and insignificant difference (65.0% in fresh vs. 80.0% in brackish). This difference in the effect of the treatments on the two stocks was not large enough to be significant: mortality level was independent of the origin of the grandparent ($G = 2.698$, *d.f.* = 1, $P > .10$).

For both males and females, fish reared in fresh water were significantly shorter (11 mm SL vs. 15 mm SL for females, $P < .001$) and lighter (18–19 vs. 58–69 mg wet weight for females, $P < .001$) than fish reared in brackish water (Table 4). Neither males nor females differed in condition in different treatments. The source of the grandparents had a significant effect only on male weight ($P < .005$); males whose ancestors came from fresh water were lighter.

The seven surviving fish which had received heavy medication did not differ either in weight ($t = 1.059$, $P > .20$) or in length ($t = 0.035$, $P > .90$) at 86 days of age from the six nonmedicated fish in treatment FF. They did differ significantly both in weight ($t = 4.042$, $P < .001$) and in length ($t = 5.124$, $P < .005$) from the fish in treatment FB. Only one of eight medicated fish died before the 86th day; this was significantly better survival than was experienced by fish in treatment FF ($G = 8.382$, $P < .005$), but was not significantly different from that experienced by fish in treatment FB ($G = 0.290$, $P >$

TABLE 4. *Length and weight of fish 86 days old. Experiment II.*

Conditions experienced by		Traits at 86 days ± 2 SE					
		Females			Males		
Grand-parents	Self	*n*	Length	Weight	*n*	Length	Weight
Fresh	Fresh	6	10.6 ± 1.2	18.0 ± 6.5	1	13.0	26.1
Fresh	Brackish	13	15.0 ± 1.1	69.3 ± 15.8	11	15.2 ± 0.6	53.3 ± 7.9
Total		19	13.6 ± 1.3	53.1 ± 15.6	12	15.0 ± 0.7	51.1 ± 8.5
Brackish	Fresh	11	10.9 ± 0.5	19.1 ± 2.7	2	12.7	29.7
Brackish	Brackish	5	14.5 ± 2.1	58.3 ± 20.7	12	15.6 ± 0.4	67.6 ± 5.2
Total		16	12.0 ± 1.1	31.4 ± 11.3	14	15.2 ± 0.7	62.2 ± 8.6

TABLE 5. *Survival, length, and weight of fish 86 days old. Experiment III. Fish from a freshwater reservoir in Hawaii.*

A. Survival

Treatment	Number at 86 days Alive	Dead	Total	% Surviving
Fresh	32	8	40	80.0
Brackish	32	8	40	80.0
Total	64	16	80	$G = 0.0, P > .99$

B. Length and Weight

	Traits at 86 Days ± 2 SE									
	Females					Males				
Treatment	*n*	Length	*P*	Weight	*P*	*n*	Length	*P*	Weight	*P*
Fresh	11	18.4 ± 1.2	.118	96.1 ± 18.2	.863	21	16.9 ± 0.6	.321	69.2 ± 5.1	.059
Brackish	13	17.3 ± 0.7		97.9 ± 10.2		19	16.5 ± 0.6		81.0 ± 11.5	
Total	24	17.8 ± 0.7		97.1 ± 9.8		40	16.7 ± 0.4		74.8 ± 6.3	

.50). None of the data from medicated fish appear in Tables 3 and 4; the medication experiment was analyzed separately as reported above.

The results of Experiment II indicated that the dramatic differences in growth rates seen in Experiment I between fish raised in fresh or in brackish water for the rest of their lives were not caused by a contaminated fresh water supply, because the difference in growth rates in Experiment II were just as dramatic, even for the medicated fish. That medicated fish had higher survival rates than unmedicated fish, even though both groups grew equally poorly, indicated that fish grown in fresh water were susceptible to disease, and that this effect could account for all the differences in survival rate of fish raised in fresh and brackish water. However, it could not account for the difference in growth rates. We suggest that fresh water affected growth, and that fish which grow poorly are more susceptible to disease, but these data do not demonstrate that.

None of the fish from Texas grew or survived well in fresh water—not even those whose parents or grandparents had been caught in fresh water. The hypothesis that best fit the data was that the fish from Armand Bayou were maladapted to the fresh water used for the laboratory experiments. One explanation, that we did not test, was that the fresh water in Texas contains factors that promote the growth and survival of *Gambusia* and that the water used for the laboratory experiments lacked these factors. Another hypothesis is that the freshwater population in the field was either swamped by gene flow from the estuary or was the result of a recent colonization event. To test this hypothesis in Experiment III, we raised fish that had been living for about 70 years in a freshwater reservoir in Hawaii under precisely the same conditions as we had raised fish in Experiment II.

Experiment III.—In Experiment III, the survival pattern changed dramatically; the fish survived equally well in fresh and in brackish water (Table 5a). Moreover, there were no significant differences in the length and weight of either males or females raised in fresh or in brackish water at 86 days of age (Table 5b). The sexes differed significantly in length ($P = .004$) and weight ($P = .001$), but not in condition ($P = .490$). However, within each sex fish were thinner in fresh water: at a given weight near the mean weight, females were 1.4 mm SL longer in fresh water than in brackish water (for both sexes, $P < .001$). Thus the laboratory freshwater supply did have measurable effects on fish known to be well-adapted

TABLE 6. *Electrophoretic data bearing on the hypotheses of local adaptation.*

A. Direct test for differences in overall distribution of alleles between fresh and brackish populations.

		6-PGD						α-GPD						
Field Site	*n*	F*	M	S	Total	G	P	F	M	M_1	S	Total	G	P
Fresh	56	5	7	100	112	9.116	.025	0	78	3	25	106	9.742	.01
Brackish	53	7	17	82	106			1	60	12	29	102		
	109	12	24	182	218			1	138	15	54	208		

B. Observed genotypic frequencies.

		6-PGD							α-GPD									
Field site	*n*	FF	MM	SS	FM	FS	MS	*n*	FF	MM	M_1M_1	SS	FM	FM_1	FS	MS	MM_1	M_1S
Fresh	56	0	0	44	0	5	7	53	0	28	0	2	0	0	0	20	2	1
Brackish	53	0	4	34	1	6	8	51	0	15	1	3	1	0	0	21	8	2
	$G = 18.99, P < .005$								$G = 24.45, P < .005$									

* F, M, M_1 and S refer respectively to an allelomorphic series ranging in relative mobility from most anodal (fastest) to most cathodal (slowest)

to fresh water, but these were subtle effects, much less dramatic than the effects of laboratory fresh water on fish from Armand Bayou.

Electrophoretic data.—To test the possibility of genetic differentiation between the two populations, we performed horizontal starch gel electrophoresis on extracts of whole ground fish from both fresh and brackish water populations, using standard methods (Selander et al., 1971; Harris and Hopkinson, 1976). A preliminary test of 20 fish from each stock for 24 structural proteins revealed (a) problems in scoring three systems (2 glutamate-oxaloacetate transaminases [GOT] and phosphoglucomutase [PGM]), which were discarded, 14 systems which could be scored but were monomorphic and identical in both populations (alcohol dehydrogenase [ADH], isocitrate dehydrogenase [ICD], 2 malate dehydrogenases [MDH], glyceraldehydephosphate dehydrogenase [GAPDH], aconitase [ACON], two dipeptidases, adenosine deaminase [ADA], and five general proteins), and (c) seven systems in which there were clear indications of electrophoretic variability (glycerol-3-phosphate dehydrogenase [α-GPD], phosphogluconate dehydrogenase [6-PGD], 3 lactate dehydrogenases [LDH], and 2 glucosephosphate isomerases [GPI]). We were unable to resolve the esterase loci described by Yardley and Hubbs (1976). We therefore increased the sample size for these seven systems; for two (6-PGD and α-PGD) there was sufficient variability to permit tests for significant differences between stocks. Data on both 6-PGD and α-GPD indicated that the two populations differed significantly in allele frequencies for the two systems but the differences were not large (Table 6a). We interpret that as indicating weak but detectable genetic isolation between the two stocks.

Both systems in both populations did not differ significantly from Hardy-Weinberg expectations (Table 6b, fresh: 6-PGD, $G = 0.25, P > .5$; α-GPD, $G = 0.62, P > .90$; brackish: 6-PGD, $G = 1.6$, $P > .5$; α-GPD, $G = 1.04$, $P > .90$). However, in both systems the genotypic frequencies differed significantly between fresh and brackish populations. Thus both allele and genotypic frequencies differ in the fresh and brackish populations. We conclude that the two populations differ genetically, but not by very much, and that further differentiation is prevented by migration or by the short period since a possible colonization event in 1967–1969. Yardley and Hubbs (1976) also reported genetic heterogeneity between mosquito fish populations over similarly short distances in Texas.

Fitness calculations.—Of course, growth and survival to 86 days of age are not the most direct measures of fitness. In

TABLE 7. *Estimates of reproductive contributions from 4 of 8 treatments. Experiment I.*

Treatment							
	Conditions experienced						
Mother	Week 1	Rest of life	n	Age at maturity	Survival to maturity (l_α)	Size of 1st brood (b_α)	$l_\alpha b_\alpha$
Fresh	Fresh	Brackish	24	170.4	.60	8.13	4.88
Fresh	Brackish	Brackish	22	171.0	.55	7.91	4.35
Brackish	Fresh	Brackish	17	159.8	.85	9.40	7.99
Brackish	Brackish	Brackish	11	162.1	.37	7.36	2.72

Experiment I, we raised all surviving fish to maturity; most relevant here are data on survival to maturity, age at maturity, and size of first brood. Because only 10 of the females raised for the rest of their life in fresh water survived to give birth, we report only the data for fish raised for the rest of their life in brackish water (n = 75), where the sample size justifies some inferences.

The data are equivocal. In the experiment, the expected number of offspring in the first brood of a newborn female, adjusting for the different mean survival rates in the different treatments, was 4.88 for FFB, 4.35 for FBB, 7.99 for BFB, and 2.72 for BBB (Table 7). Ages at maturity were greater for females whose parents came from fresh water, but not significantly so (170.7 days vs. 161.5 days, P = .506). Broods (adjusted for size of female) were slightly smaller for females whose parents came from fresh water, but again not significantly so (8.02 vs. 8.69, P = .385). The variability among replicates and the low survival rate (.37) in the BBB treatment, which occurred between 86 days and maturity, contributed to the lack of a significant difference in reproductive fitness between stocks.

Overall discussion.—One inference from these experiments is that all the fish found at Armand Bayou, regardless of whether they were found in fresh or in brackish water, were maladapted to life in fresh water: they survived less well and attained smaller sizes at a given age than fish raised in brackish water. Of the 100 fish still alive in freshwater at 86 days of age, only 10 females survived to give birth at 147–207 days of age. Nevertheless, the freshwater population in Texas appeared to be thriving: fish were numerous and pregnant females were common. We therefore conclude that laboratory conditions mimicked the direction but not the degree of the impact of fresh water in the field: fresh water had a much stronger impact in the laboratory. The importance of the effect in the field populations cannot be discounted, especially in view of the dramatic difference between Experiment II and Experiment III. Thus the primary explanation for the differences between fresh and brackish populations noted in Table 1 is physiological, not evolutionary or developmental. This result does not rule out microgeographic adaptation or some developmental sensitivity to early environment as partial explanations for the pattern in the field data, but it does relegate them to a minor role. The electrophoretic data are irrelevant, suggesting weak but detectable genetic isolation of stocks, which is consistent with either genetic or physiological explanations.

An alternative explanation of the results is that the laboratory fresh water lacked growth-promoting factors found in fresh water in the field in Texas, and present—or compensated for by other factors—in Instant Ocean. If that is the case, one then has to explain why the fish from Hawaii, whose ancestors came from within 5 km of the Texas stocks, did not need this factor. With the evidence in hand, this hypothesis cannot be ruled out.

Suppose the fish were maladapted to fresh water. What then? *Gambusia* is usually found in fresh water and does not enter water more saline than 20‰ (Simpson and Gunter, 1956). We suggest only two hypotheses are tenable: either fish movement into the freshwater stream at times of high water is providing enough gene flow to prevent local adaptation, or the freshwater population is the result of a recent colonization event, probably since 1969, following road construction in the area. Both processes could have occurred together. In either case, the fish living in brackish water have lost, or never gained, the tolerance of fresh water exhibited by the species over most of its range. That conclusion leads us to the following prediction: if the freshwater population of this study were isolated from the brackish population by a weir, it should either go extinct or measurably increase in tolerance to fresh water.

If we assume, for purposes of discussion, that the freshwater population is both marginal and maladapted, then Carson's (1955) and Soulé's (1973) discussions of the genetics of marginal populations are relevant. Carson noted that marginal populations of *Drosophila* are more homozygous for chromosomal polymorphisms than central populations. He interpreted this as an adaptation for higher recombination rate in marginal populations. Soulé suggested that marginal populations of vertebrates were deficient in allelic polymorphisms because they are less vagile than *Drosophila,* and that marginal populations lose alleles through genetic drift and reduced immigration. In this case, the freshwater population shows a tendency towards fixation of the commonest allele for both 6-PGD and α-GPD (Table 6a), but we would add to Soulé's interpretations another alternative hypothesis: selection favors the common alleles in fresh water. The data are not adequate to discriminate among drift, selection and other possibilities (Lewontin, 1974), nor are the differences in allele and genotypic frequencies so large as to call forcefully for explanation.

If we—again for purposes of discussion—conceive of the freshwater population as in a dynamic equilibrium between selection (for local adaptation) and immigration (from the estuary), then the appropriate theoretical model is an island, or pocket, with a small population receiving immigrants from a large, central population. Haldane (1930) analyzed the case where one allele is favored locally, but the other is continually introduced by immigrants. He found that the deleterious allele will be kept at low frequencies so long as m, the migration coefficient, is sufficiently smaller than s, the selection coefficient. Nagylaki's (1975) results are similar. If selection and migration are the only forces at work in this case, significant but not dramatic differences in allele frequencies (cf. Table 6) suggest that they are of the same magnitude, with a selection coefficient (s) slightly larger than the migration coefficient (m). Haldane (1930), Hanson (1966), and Nagylaki (1975) all predict a sudden collapse in local adaptation when migration exceeds a certain threshold (cf. Felsenstein, 1976). The electrophoretic data suggest that the balance between selection and migration is such that this threshold has not been exceeded for 6-PGD and α-GPD; the data on growth and survival suggest that this threshold has been exceeded for loci associated with osmoregulation.

SUMMARY

At Armand Bayou, Texas, mosquito fish (*Gambusia affinis*) living in a small, freshwater stream had fewer, larger young and made smaller reproductive efforts than mosquito fish living 150–300 m away in a large, brackish estuary. In Experiment I, we raised the progeny of field-caught fish from both fresh and brackish water half in fresh and half in brackish water for the first week of life, and then half in fresh and half in brackish water for the rest of their life, at densities of 20 fish per tank (eight treatments: FFF, FFB, FBF, FBB, BFF, BFB, BBF, BBB). The major effects were all caused by the environment in which the fish were raised after the first week of life: fish raised in fresh water had lower survival (51.6% vs. 93.6%), were shorter (15.0 vs. 17.2 mm SL), and were lighter (72.5 vs. 118.2 mg wet weight) at

86 days of age than fish raised in brackish water.

In Experiment II, we reared the progeny of fish from treatments FBB and BBB in isolation from birth in either fresh or in brackish water made up from distilled water (four treatments: FF, FB, BF, BB). Eight of the fish reared in fresh water also received medication. Fish reared in fresh water again had lower survival (46.5% vs. 79.5%), were shorter (11.1 vs. 15.2 mm SL), and were lighter (20.2 vs. 63.2 mg wet weight) than fish reared in brackish water. The fish that had received medication did not differ in length and in weight from the other fish reared in fresh water, but they did have higher survival rates.

In Experiment III, we reared the progeny of fish from a freshwater reservoir in Hawaii in fresh or in brackish water. The results differed strikingly from the results of the first two experiments. Fish reared in fresh water had the same survival rate (80.0% vs. 80.0%), were the same length or slightly longer (17.4 vs. 16.8 mm SL), and weighed the same or slightly less (78.4 vs. 87.9 mg wet weight) than the fish reared in brackish water.

The fresh and brackish stocks differed significantly in the distribution of allele frequencies at 2 of 24 loci examined (6-PGD and α-GPD); these differences were small. We suggest that the primary cause of the differences noted in the life-history traits of fresh and brackish populations in the field was not local adaptation (an evolutionary explanation) or developmental sensitivity to early environment (an ontogenetic explanation) but maladaptation to the freshwater environment (a physiological explanation).

ACKNOWLEDGMENTS

Roy Johnson and John Key of the Texas Parks and Wildlife Laboratory at Seabrook, Texas, gave cheerful and energetic help in the field. Phil Schmitz, Maureen Longworth, Deborah McGann, and Dan Hannah assisted in the experiments. Dave Wake, Jim Patton, Mike Hirshfield, Ray Huey, Peter Frank, Henry Schaffer, and two anonymous reviewers offered comments that considerably improved the manuscript. This study was inspired by a question Bill Wellington asked Stearns during his Ph.D. defense at the University of British Columbia in 1975. It was supported by the American Philosophical Society and the Miller Institute for Basic Research in Science. The National Science Foundation provided funds for the electrophoretic studies through grant DEB 72-02545.

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