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GENETIC AND DEMOGRAPHIC RESPONSES OF MOSQUITOFISH (*GAMBUSIA HOLBROOKI* GIRARD 1859) POPULATIONS STRESSED BY MERCURY

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Abstract—Genetic and demographic changes in mosquitofish populations are reported after chronic (111 d) exposure to mercury. Sex ratios, normally female-biased in field populations, were also female-biased in control mesocosms. However, the sex ratio was male-biased in the mercury treatments. Frequencies of glucosephosphate isomerase-2 (*Gpi-2*) allozymes for fish exposed to mercury differed from initial frequencies and from those of control fish. In a selection-component analysis, female sexual selection was statistically significant for the mercury-treated fish; the proportion of females that were gravid differed among *Gpi-2* genotypes. The number of developing embryos per female also differed among *Gpi-2* genotypes. Mercury had genotype-specific effects on mosquitofish reproduction in addition to genotype-specific effects on mortality reported earlier. These effects may reflect metabolic qualities of the *Gpi-2* genotypes or loci closely linked to the *Gpi-2* locus.

Keywords—*Gambusia holbrooki* Fish Mercury Genetics Demography

INTRODUCTION

Toxicant stress to individuals can modify genetic characteristics of populations. If populations are composed of individuals that vary in their tolerance to a toxicant, and this tolerance has a genetic basis, tolerant genotypes should increase in frequency as stress continues. Tolerance of a toxicant can be manifest as differential viability, growth, or reproduction.

Differences in individual tolerance during toxicant stress have been linked to physiological [1,2], behavioral [3], and genetic [4,5] mechanisms. Mechanisms of resistance to metals described to date are all energy-demanding (e.g., metallothionein production [6,7], granule production [4,8], or changes in uptake or elimination rates [4]). Energy expended on resistance activities is not available for other activities such as growth or reproduction. Chronic stress is accompanied by changes in an organism's energy budget as energy is allocated to resistance [9]. Therefore, changes in individual growth and reproductive activity are key indicators of susceptibility to sublethal levels of toxicants.

Environmental stress is often used to assess relative fitness differences (e.g., survival, growth, or fecundity) among allozyme variants [10,11]. Changes in allozyme frequencies have been suggested as indicators of toxicant stress in natural populations [12,13]. Koehn [14] argued that allozyme polymorphisms for single genes can have measurable effects on physiological performance and ultimately on adaptation. Such physiological differences can be reflected in differences in individual growth, fecundities, timing of reproduction, behavior, or other critical aspects of life history. Many studies indicate a trade-off between growth or reproduction and survival in toxicant-stressed environments, and a relationship between individual performance and allozyme genotype. For example, Frati et al. [15] argued from studies of the soil arthropod, *Orchesella cinta*, that exposure to metals may impact specific loci but not overall genetic variability. For *O. cinta*, they observed a correlation between metal tolerance and frequencies of allozymes of glutamate oxaloacetate transaminase (Got).

We recently demonstrated differential survival in mosquitofish (*Gambusia holbrooki*) during acute exposures to mercury. Median times-to-death varied significantly among fish of different allozyme genotypes, sizes, and sexes [16,17]. Smaller fish and male fish had earlier times-to-death than did larger or female fish. Additionally, survivorship was related to allozyme genotype at the glucosephosphate isomerase-2 (*Gpi-2*) locus. Mosquitofish of the genotype $Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$ died sooner than fish of other *Gpi-2* genotypes [16,17]. Significant differences in glycolytic and Krebs cycle metabolite pools were also found among *Gpi-2* genotypes acutely exposed to mercury [18,19]. Differences in pools of lactic acid and glucose-6-phosphate between mosquitofish homozygous for the $Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$ genotype and the other genotypes suggested that these homozygotes had a higher rate of metabolite turnover during mercury exposure. This higher turnover may be linked to their earlier times-to-death. The hypothesis of differential metabolite turnover is further supported by recent work demonstrating that earlier times-to-death for $Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$ homozygote did not result from

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more rapid inactivation of the *Gpi-2*^{38}/*Gpi-2*^{38} form of the enzyme relative to the enzyme forms produced by the other genotypes [20]. This linkage of *Gpi-2* genotype-specific sensitivity to central metabolic pathways suggested that components of fitness other than survival—e.g., sexual selection, fecundity, and growth—could also be influenced by the *Gpi-2* genotype.

The present study extends our understanding of factors influencing mercury susceptibility of mosquitofish to potential effects on reproduction and growth during chronic exposure. Specifically, effects on reproduction and growth were examined for individuals from control and mercury-exposed populations relative to allozyme genotype (especially *Gpi-2*).

METHODS

Mesocosms

Six 7,250-L pools were filled with water from Upper Three Runs Creek, a soft water stream on the Department of Energy's Savannah River Site near Aiken, South Carolina [21]. Water quality of Upper Three Runs Creek has been described [16]. Each pool contained several clusters of plastic strips (5 × 70 cm) anchored to the pool bottom to provide juvenile mosquitofish with refuge from cannibalistic adults. Approximately 25% of each pool surface was shaded with nursery screening. Pools also had Styrofoam floats to provide shade. Water levels were maintained by precipitation and the addition of well water as needed.

Two control pools contained unspiked water (<0.1 μg/L of mercury). Mercury was added weekly as $HgCl_2$, to two pairs of pools to realized concentrations of 18 μg/L and 42 μg/L, respectively. Water samples (acidified with 0.5 ml distilled nitric acid per 500 ml) were taken for mercury analysis before weekly additions and within 1 h of each addition. Analysis of total reactive mercury was done using a Perkin-Elmer (Norwalk, CT) 50A atomic absorption spectrophotometer. Water samples for determination of pH, temperature, and other water quality parameters were also taken from each pool weekly.

Fish populations

Mosquitofish were obtained from Risher Pond, a 1.1-ha farm pond also on the Savannah River Site. This pond had a soft water chemistry similar to that of Upper Three Runs Creek [21] and has no known history of mercury pollution. Collections were made over a 5-week period to obtain the necessary numbers of fish. Fish were collected by dip net or seine and transported in insulated containers of pond water treated with Stresscoat™ (Aquarium Pharmaceuticals, Chalfont, PA). Fish were maintained immediately after collection in a 520-L tank (Living Stream™ model LSW-700; Toledo, OH) until they could be distributed among the pools. There was negligible mortality of fish during this holding period.

Fish were randomly assigned to the six pools. Nine hundred eighty-five fish were used to initiate each pool. After fish were allocated to the six pools, 90 were randomly removed from each. These 540 fish were used to define the initial demographic and genetic qualities of the pool populations. Fish were fed daily (4.5 g/pool) with Tetramin® fish flakes (Tetrawerke, Germany). Additionally, there was a natural growth of algae and microinvertebrates on which the fish could feed. Nitrate (KNO_3 to 0.08 mg N/L) and phosphate (K_2HPO_4 to 0.08 mg P/L) salts had been added 3 weeks before the addition of fish to foster algal growth. Fish were placed into the pools from February 13 to April 6, 1992. Spiking of pools with mercury began on April 9, 1992.

At the termination of the experiment (111 d), all fish were weighed and measured for standard length. Fish in the final samples were classified as mature adults (females by a dark spot on the abdomen and males by the presence of a gonopodium) or juveniles. Adults consisted of fish born during the course of the experiment and individuals that survived since the populations were established. All juvenile fish had spent their entire lives in the experimental pools. Females were dissected to determine the number of eggs and developing embryos. All individuals and one embryo from each gravid female were taken for electrophoretic analysis.

Fish growth

Size-at-age data from a sample of fish taken from control ($N = 45$ from each replicate) and mercury-treatment ($N = 35$ from each replicate) pools were compared to evaluate effects of mercury treatments on growth. Age estimates were obtained from each specimen using counts of daily growth increments on sagittal otoliths [22]. Otoliths were obtained from crania that were removed from fish carcasses prior to their use for electrophoresis. Otoliths were prepared according to Haake et al. [23], resulting in a thin otolith section containing the core embedded in epoxy [24]. Two independent counts of growth increments were made for each otolith; a third count was made if these differed by more than 10%. Counts of otolith increments were judged to represent fish age because results of the laboratory experiment described below indicated that otolith increments formed in juvenile fish daily.

In a verification experiment, progeny from several mosquitofish were reared in the laboratory from hatching and sacrificed over a 90-d period. Otoliths from these specimens were prepared as described above and growth increments enumerated. A regression of those counts of otolith increments on the number of days that a fish had been reared was significant and had a slope of 0.97. This slope was not different from 1 ($t_{32} = 1.27$, $p = 0.22$); thus, we concluded that growth increments did form daily in fish experiencing conditions sufficient for growth. Whether or not mercury treatment disrupted increment formation and caused a bias in growth estimates was not assessed. If mercury treatments disrupted the daily patterns of increment formation, as has been observed with some other stressors [25], then age of those fish may be underestimated and growth overestimated. However, there was no evidence of such disruption noted during otolith examination and data analyses.

Electrophoresis

Electrophoretic methods were those described in previous reports of mosquitofish response to toxicants [16,17,26]. The following enzymes were assayed for all adult and juvenile fish: adenosine deaminase (Ada, E.C. 3.5.4.4), fumarate hydratase (Fh, E.C. 4.2.1.2), glucosephosphate isomerase-2

(Gpi-2, E.C. 3.5.1.9), isocitrate dehydrogenase-1,2 (Icd-1,2, E.C. 1.1.1.42), malate dehydrogenase-1 (Mdh-1, E.C. 1.1.1.37), mannosephosphate isomerase (Mpi, E.C. 3.5.1.8), leucylglycylglycine-peptidase (lgg-Pep, E.C. 3.4.−.−) and phenylalanylproline-peptidase (pp-Pep, E.C. 3.4.−.−). These enzymes also were assayed for embryos except that there was no detectable Mdh-1 activity in embryos.

Data analysis

Statistical analyses of water chemistry, fish growth, and demographic data were carried out with the Statistical Analysis System [27] using analysis of variance and analysis of covariance with standard length as a covariate. Duncan multiple-range tests were used to identify differences among pools. The Dunn–Šidák adjustment was used to adjust experiment-wise error rate [28]. Allozyme and genotypic frequencies, mean heterozygosities, and fit of observed data to Hardy–Weinberg expectations were calculated using BIOSYS-1 [29]. Rare alleles were pooled for *Icd-1* and *Gpi-2* to test for fit to Hardy–Weinberg expectations. Contingency χ^2 tests were used to test for homogeneity of genotypic distributions for fish among pools and the initial sample.

Selection-component analyses (SCA) were done using parent–offspring combinations [30]. Allozyme genotypes were obtained for adult males, nongravid females, gravid females, and a single embryo from each gravid female. Rare genotypes were pooled prior to analysis. The sequence of hypotheses was tested with the underlying null hypothesis of no selection. Fecundity selection was tested using data on the number of offspring from each female of each genotype and the null hypothesis that the number of offspring per female was not related to genotype.

RESULTS

Water quality was similar in the control and mercury-treatment pools except for mercury concentrations. Average mercury concentrations 1 h after spiking were 18 μg/L and 42 μg/L for the low and high mercury treatments, respectively, and <0.1 μg/L in the control pools. Within 2 to 3 d following weekly mercury additions, the concentrations measured in the water dropped exponentially to ambient levels (<0.1 μg/L) due to the rapid adsorption of mercury to solid surfaces. No fish survived past 2 months in the high concentration pools. Consequently, the remaining two pairs of pools were examined as simply control and mercury treatments.

Significant differences occurred among pools in the total number of fish, mean sizes, and ratios of sex and age (Table 1). The mercury-treated populations were more similar for these characteristics than were the control pools. The total number of fish harvested was 3,904 from the control pools and 1,341 from the mercury-treated pools a population increase of more than two times for the control and a decline in total number for the mercury treatments. The age ratio was significantly different among pools, but there was no consistent relationship between life history stage and treatment. The proportion of the population classified as juveniles was 24 and 81% in control pools A and B, respectively, and 54 and 72% in mercury-treated pools A and B, respectively. There was a highly significant difference in sex ratio between treatments (Table 1). Sex ratios were strongly female-biased in the control pools but were male-biased in the mercury-treated pools.

Mean standard lengths for adult male mosquitofish were significantly greater in the mercury-treated pools than in control pools (Table 1). Standard length for females in mercury-treatment A were greater than that of females in both control treatments, and females in mercury-treatment B had greater standard length than did those of control B. Wet weight of fish varied greatly among pools and showed no consistent pattern with respect to mercury treatment.

Significant differences in growth characteristics for juvenile fish ≤50 d old and adult males were observed between the pool populations of mosquitofish; however, these differences were not consistent with treatment status (Table 2). Mean juvenile growth rate differed significantly between the two control pools ($p < 0.05$) for weight and length. Juve-

Table 1. Characteristics of mosquitofish in control and mercury-treated pools

	Control				Mercury-treated			
	Pool A		Pool B		Pool A		Pool B	
Parameter	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD
Standard length (mm)								
Females	415	42.6 ± 3.6 B,C[a]	388	42.0 ± 4.0 C	123	44.1 ± 3.9 A	101	43.0 ± 3.8 B
Males	163	24.4 ± 2.3 B	111	24.4 ± 3.3 B	150	26.0 ± 2.3 A	143	25.6 ± 3.0 A
Juveniles	710	8.6 ± 0.8 C	2,117	10.5 ± 2.4 A	506	9.7 ± 1.3 B	318	104 ± 1.8 A
Weight (mg)								
Females	415	1,835 ± 494.4 A	388	1,753 ± 558.1 A,B	123	1,800.9 ± 561.4 A	101	1,670.0 ± 483.0 B
Males	163	245.7 ± 66.5 B	111	250.4 ± 132.4 B	150	282.0 ± 84.6 A	143	268.5 ± 125.9 A,B
Juveniles	710	14.3 ± 5.2 D	2,117	26.3 ± 19.2 A	506	19.0 ± 6.7 C	318	24.5 ± 14.0 B
Age ratio (adult:juvenile)		0.81:1		0.24:1		0.54:1		0.72:1
Sex ratio (female:male)		2.55:1		3.50:1		0.82:1		0.71:1

[a]Pool means with the same capital letter are not significantly different (Duncan multiple-range test).

Table 2. Growth information for juvenile and male mosquitofish and female reproductive characteristics in control and mercury-treated mesocosms

	Control		Mercury-treatment	
	Pool A	Pool B	Pool A	Pool B
Growth	(27)[a]	(29)	(27)	(26)
Weight (mg/d)				
Juvenile	0.0008 A	0.0005 B	0.0007 A	0.0007 A
Males	0.0031 A	0.0021 B	0.0022 B	0.0020 B
Length (mm/d)				
Juvenile	0.370 A	0.335 B,C	0.343 B	0.321 C
Males	0.339 A	0.260 B	0.226 C	0.236 C
Reproduction	(414)	(337)	(121)	(100)
% Gravid	63.6	60.7	56.6	63.4
Total eggs plus embryos per female	52.2 A	33.4 C	48.6 B	35.3 C
No. embryos per female	28.8 A	24.2 A,B	20.6 B,C	16.6 C

[a]Number in parentheses are sample sizes. Means with the same capital letter are not significantly different.

nile growth rates, for weight, were similar in the mercury-treated pools ($p = 0.84$) (Table 2). Mean juvenile daily length increase for the mercury treatments was 0.332 ± 0.036 mm/day, similar to control pool B. The relationships between standard length and age were highly significant ($r^2 = 0.82$ for controls and $r^2 = 0.94$ for mercury treatments) with all fish considered (Fig. 1).

There were no differences between treatments in the percent of females gravid nor the total reproductive effort (eggs + embryos/female) (Table 2). However, there was a significant difference between treatments in the number of developing embryos per female. Females in the mercury treatment had significantly fewer developing embryos (18.8/female) than did those in the control pools (26.7/female) ($p < 0.01$). Although embryos were examined for gross abnormalities such as those described by Weis et al. [31] as they were counted, only two gross abnormalities were observed among the 24,315 embryos surveyed.

Allozymes were in Hardy–Weinberg equilibrium frequencies. Most loci showed no change in allele frequency over the course of the study, and allozyme frequencies were essentially the same for initial, adult, and juvenile fish samples (Table 3). No significant differences in mean heterozygosity were observed between initial and final samples nor between treatments. The *Gpi-2* locus was the exception. There was a general decrease in the frequency of the $Gpi\text{-}2^{100}$ allele from the initial frequency ($p_{100} = 0.52$) to mercury-treated adults ($p_{100} = 0.52$ and 0.50 in pools A and B, respectively) and mercury-treated juveniles ($p_{100} = 0.45$ and 0.43 in pools A and B, respectively). In contrast, no changes in $Gpi\text{-}2^{100}$ allele frequencies were noted in control pools A ($p_{100} = 0.53$) and B ($p_{100} = 0.52$). There was a lower frequency of $Gpi\text{-}2^{100/100}$, $Gpi\text{-}2^{100/66}$, and $Gpi\text{-}2^{100/38}$ genotypes among adults in the mercury-treated pools relative to the juvenile fish in these pools (Fig. 2). In contrast, the $Gpi\text{-}2^{66/38}$ and $Gpi\text{-}2^{38/38}$ genotypes were more common among adults than among juveniles in the control pools.

Selection-component analyses were performed for loci with sufficient data for all classes, i.e., *Gpi-2*, *Icd-1*, *Icd-2*, and *Mpi*. For the SCA (Table 4), only the first significant deviation is discussed because hypotheses are interdependent with the sampling design used. No significant components were identified for *Icd-1* or *Mpi*. The selection-component hypothesis for female gametic selection showed a significant effect for control pool A ($p = 0.003$) and a marginally significant value for control pool B ($p = 0.07$) for the *Icd-2* lo-

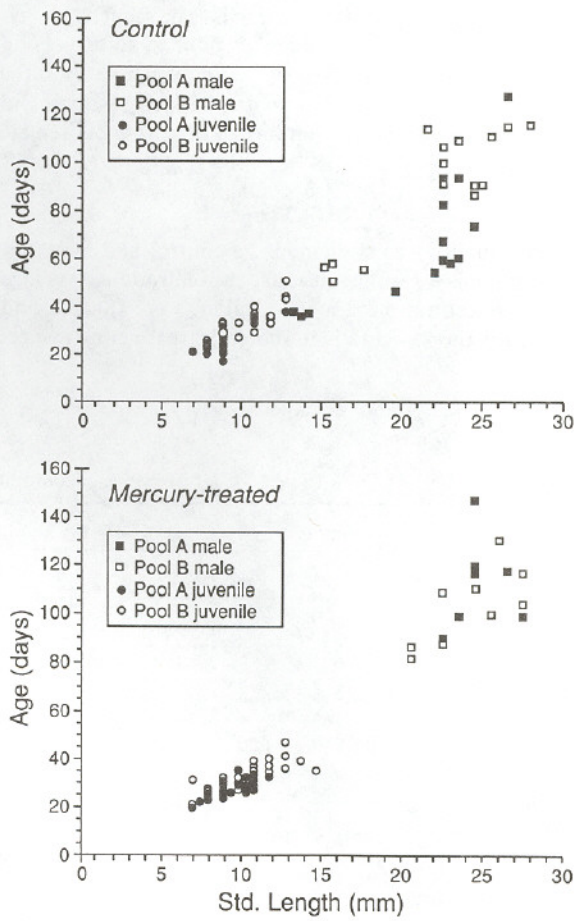

Fig. 1. Size and age relationships for control and mercury-treated mosquitofish. Circles denote juveniles, and squares denote males.

Table 3. Allele frequencies for the initial sample and at the termination of the mesocosm treatments

Locus/Allele	Initial Sample	Control				Mercury-treated			
		Adult		Juvenile		Adult		Juvenile	
		A	B	A	B	A	B	A	B
Adenosine deaminase									
Ada^{100}	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Ada^{88}	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Fumarate hydratase									
Fh^{100}	0.98	0.97	0.97	0.99	0.98	0.96	0.97	0.97	0.97
Fh^{118}	0.02	0.03	0.03	0.01	0.02	0.04	0.03	0.03	0.03
Glucosephosphate isomerase-2									
$Gpi\text{-}2^{100}$	0.52	0.53	0.52	0.55	0.52	0.52	0.50	0.45	0.43
$Gpi\text{-}2^{66}$	0.35	0.34	0.34	0.37	0.36	0.33	0.38	0.42	0.41
$Gpi\ 2^{38}$	0.13	0.13	0.14	0.08	0.12	0.15	0.12	0.13	0.16
Isocitrate dehydrogenase-1									
$Icd\text{-}1^{134}$	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.06	0.09
$Icd\text{-}1^{116}$	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02
$Icd\text{-}1^{100}$	0.92	0.91	0.91	0.91	0.91	0.91	0.90	0.91	0.89
Isocitrate dehydrogenase-2									
$Icd\text{-}2^{161}$	0.25	0.23	0.23	0.20	0.26	0.24	0.24	0.29	0.22
$Icd\text{-}2^{100}$	0.75	0.77	0.77	0.80	0.74	0.76	0.76	0.71	0.78
Malate dehydrogenase-1									
$Mdh\text{-}1^{118}$	0.26	0.28	0.27	0.26	0.26	0.26	0.27	0.28	0.22
$Mhdd\text{-}1^{100}$	0.74	0.72	0.73	0.74	0.74	0.74	0.73	0.72	0.78
Mannosephosphate isomerase									
Mpr^{109}	0.25	0.28	0.25	0.23	0.23	0.26	0.30	0.24	0.27
Mpi^{100}	0.75	0.72	0.75	0.77	0.77	0.74	0.70	0.76	0.73
Leucylglycylglycine-peptidase									
$lgg\text{-}Pep^{123}$	0.18	0.14	0.16	0.18	0.15	0.14	0.16	0.18	0.18
$lgg\text{-}Pep^{100}$	0.82	0.86	0.84	0.82	0.85	0.86	0.84	0.82	0.82
Phenylalanylproline-peptidase									
$pp\text{-}Pep^{100}$	0.96	0.97	0.96	0.97	0.97	0.96	0.96	0.96	0.96
$pp\text{-}Pep^{91}$	0.04	0.03	0.04	0.03	0.03	0.04	0.04	0.04	0.04
N	538.9 (1.5)	568.1 (3.2)	510.6 (0.7)	704.0 (2.3)	1162.7 (20.9)	261.7 (0.7)	243.3 (0.3)	490.1 (10.4)	309.7 (6.1)
H_{obs}	0.258 (0.67)	0.241 (0.060)	0.246 (0.06)	0.245 (0.062)	0.243 (0.063)	0.254 (0.065)	0.249 (0.0640)	0.260 (0.063)	249 (0.062)

Control, no $HgCl_2$ added. Mercury-treated, weekly additions of $HgCl_2$ to realized concentration of 18 $\mu g\ L^{-1}$. For the terminal sample, fish were classified as adult or juvenile. N = mean sample size per locus. H_{obs} = observed mean heterozygosity.

cus. This suggested selection among female gametes since heterozygous females did not yield the predicted 1:1 ratio of homozygous:heterozygous offspring for the *Icd-2* locus. For fish in the mercury treatment, *Gpi-2* showed an effect associated with female sexual selection ($p = 0.01$ and $p = 0.09$ for mercury pools A and B, respectively). Females of different *Gpi-2* genotypes differed in their reproductive traits. Among control females, approximately 70% were gravid regardless of genotype. For the mercury treatment, 43% of females homozygous for the $Gpi\text{-}2^{100}/Gpi\text{-}2^{100}$ genotype were gravid while approximately 70% of females of other genotypes were gravid at the time of collection. The fecundity of all females was reduced in the mercury treatments relative to the controls (Fig. 3). Additionally, $Gpi\text{-}2^{100}/Gpi\text{-}2^{100}$ homozygotes had significantly fewer developing embryos ($p = 0.01$) than other *Gpi-2* genotypes. Although fecundity is correlated with size in female mosquitofish, analysis of covariance that included mercury treatment, fish standard length, and the interaction between treatment and standard length indicated a significant effect ($p = 0.02$) of mercury treatment after accounting for the confounding effects of standard length.

DISCUSSION

More adults in the mercury-treated pools were survivors of the initial starting population than in the control pools. This is supported by the observation that the mean standard

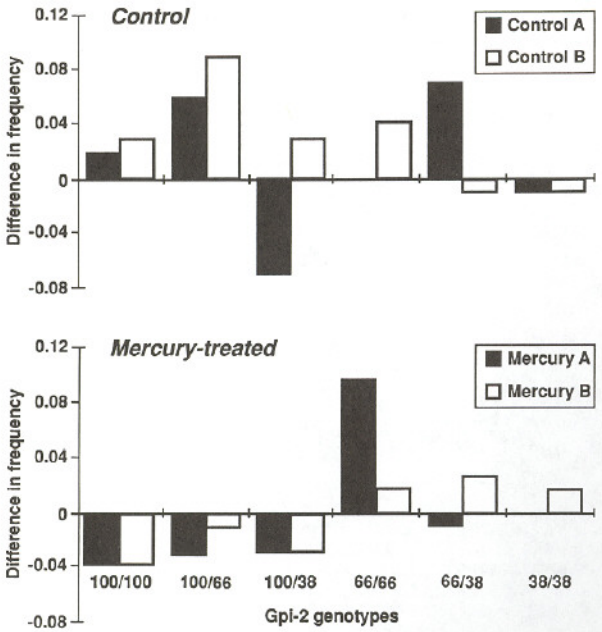

Fig. 2. Difference in *Gpi-2* genotype frequency between juvenile and adult mosquitofish. Shown is frequency in juveniles minus frequency in adults.

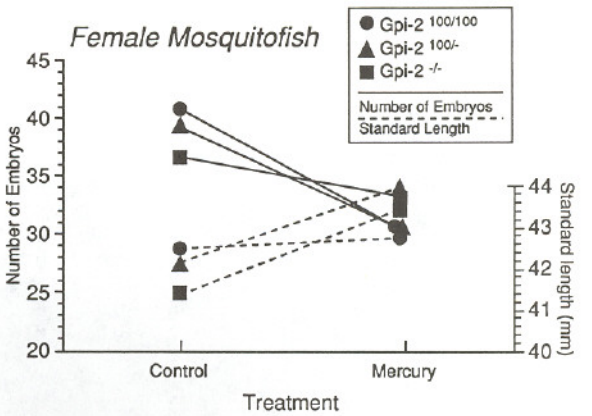

Fig. 3. Mean number of developing embryos (solid line) and standard length (dashed line) for female mosquitofish of different *Gpi-2* genotypes from control and mercury-treated pools.

length of adult mosquitofish was significantly greater in the mercury-treated pools, especially relative to control pool B. Male mosquitofish display determinate growth with arrested growth at sexual maturity [32]. Females have indeterminate growth and continue to grow after sexual maturity. Consequently, they grow larger than males. Differences likely reflected differences in mortality or, perhaps, growth between the mercury-treated pools and the controls. However, size/age relationships did not differ between treatments. One possibility is that fish in the mercury-treated pools were bigger because the smaller adults tended to die more readily than did the larger adults. We have demonstrated lower survivorship for smaller size fish in acute mercury, arsenic, and sodium chloride exposures [16,17,26,33,34]. Poor recruitment has also been reported for young white suckers exposed to toxic metals [35,36]. Sublethal toxicity affected condition, growth, and reproduction of these fish. For the mercury-treated pools the population decreased approximately 25% below the number of fish initially added to the pools. In contrast, the total number of fish in the control pools increased by more than two times the initial number.

Significant differences existed between treatments in female reproductive characteristics. Although control and mercury-treated fish had the same average total reproductive effort (eggs + embryos/female), females in the mercury treatments had significantly fewer developing embryos than did control females. Fewer developing embryos in females from mercury treatments could result because eggs are not fertilized and, therefore, do not develop, or fertilized eggs fail to develop.

Table 4. Probability values for chi-square tests for components of selection in the allozyme frequency data following the method of Christiansen et al. [30]

		Gpi-2				Icd-2			
		Control		Mercury-treated		Control		Mercury-treated	
Test	Hypothesis	A	B	A	B	A	B	A	B
T1	Female gametic selection	0.54	0.64	0.07	0.71	0.003	0.07	0.28	0.61
T2	Random mating	0.76	0.96	0.51	0.91	0.77	0.99	0.92	0.89
T3	Male reproductive selection	0.70	0.88	0.07	0.73	0.78	0.72	0.60	0.84
T4	Female sexual selection	0.55	0.52	0.01	0.09	0.05	0.06	0.51	0.63
T1 + T2 + T3 + T4	All gametic and sexual components	0.76	0.85	0.01	0.54	0.02	0.18	0.71	0.91
T5	Zygotic selection equal in sexes	0.54	0.18	0.009	0.26	0.33	0.04	0.15	0.87
T6	Zygotic selection	0.68	0.19	0.42	0.58	0.010	0.90	0.54	0.95
T5 + T6	All zygotic components	0.62	0.16	0.02	0.35	0.03	0.10	0.21	0.95
TO	Total fit to random mating and neutrality	0.78	0.61	0.001	0.51	0.004	0.09	0.55	0.98

For *Gpi-2* and *Icd-2* the less common alleles were pooled prior to analyses.

For a large, randomly mating population with no selection, the prediction is that allele frequencies will not differ among age classes. Consistent with this expectation, there was no change in the allozyme frequency for the control pools. In contrast, the change in frequency for *Gpi-2* allozymes in the mercury treatment implies selection associated with mercury exposure. For the mercury-treated pools, there was a consistent decline in the frequency of the common allozyme ($Gpi\text{-}2^{100}$). This observation was reinforced by selection-component analysis that indicated a significant effect associated with female sexual selection for the *Gpi-2* locus in the mercury treatments. Females homozygous for the common allele ($Gpi\text{-}2^{100}/Gpi\text{-}2^{100}$) were less likely to be gravid and had fewer developing embryos than other *Gpi-2* genotypes in mercury treatments and all *Gpi-2* genotypes in the control pools. These two observations suggest a mechanism for the change in *Gpi-2* allele frequency from the initial to the final juvenile samples.

Heterozygotes and homozygotes for the $Gpi\text{-}2^{38}$ and $Gpi\text{-}2^{66}$ alleles were more likely to be gravid and had more embryos per clutch than did females homozygous for the $Gpi\text{-}2^{100}$ allele. Such differential reproduction could have produced the observed change in allozyme frequency.

What mechanisms might account for a relationship between *Gpi-2* allozyme phenotype and performance of mosquitofish experiencing mercury stress? Allozymes might have different kinetic properties. Differences in the metabolite pools among *Gpi-2* genotypes have been demonstrated for mosquitofish exposed to mercury [18,19]. In acute mercury exposures, the most sensitive homozygous genotype for *Gpi-2* ($Gpi\text{-}2^{38/38}$) was found to have a distinct profile of glycolytic and Krebs cycle metabolites relative to the other *Gpi-2* genotypes [18]. Also, *Gpi-2* might be linked to a gene or genes associated with susceptibility to mercury (genetic "hitchhiking"). Although it is not yet possible to distinguish between differences due to a locus under study (*Gpi-2*) and closely linked (hitchhiking) loci, a growing body of information indicates that for mosquitofish, allozyme polymorphism for *Gpi-2* (or closely linked loci) is related to performance differences in stressful conditions. The *Gpi-2* locus has consistently shown a relationship with mosquitofish performance in four acute mercury exposures and this chronic exposure. The *Gpi-2* genotype effects differed among studies; $Gpi\text{-}2^{38/38}$ was most sensitive to acute mercury stress and $Gpi\text{-}2^{100/100}$ was most sensitive to chronic mercury stress. Heagler et al. [17] observed a relationship between *Gpi-2* allozyme frequency and mercury contamination in field populations. The $Gpi\text{-}2^{38}$ allozyme declined in frequency in samples taken from contaminated areas.

The relationship between *Pgi* (=*Gpi*) genotypes of mosquitofish has also been examined during exposure to pesticides (*Gambusia affinis*) [37] and thermal stress (*G. holbrooki*) [38]. Hughes et al. [37] reported differences in response with pesticide treatment and suggested that this relationship was due to linkage of *Pgi* loci with one or more loci affected by chlorpyrifos. Mulvey et al. [38] found that mosquitofish homozygous for the $Gpi\text{-}2^{38}$ allele had significantly longer predicted median time-to-maturity than did others when reared under thermally elevated conditions. Thus, different *Gpi-2* genotypes may perform better in different selection regimes—e.g., acute versus chronic, or warm versus cool. The variety of studies in which *Gpi-2* has been related to mosquitofish response differences suggests that *Gpi-2* genotypes, or very closely linked loci, contribute to the differences in performance observed in the mercury-treated and control populations.

Introduction of a toxicant such as mercury to the environment of mosquitofish represents a significant change in the selective regime; thus, relative fitnesses differ between control and mercury-treated environments. In both environments, however, individuals must allocate available energy and resources to maintenance, growth, and reproduction. As the fish used in this study had no previous exposure to mercury, variability in individual response reflects the existing variability of these mosquitofish and is not related to metal tolerance per se. That the mercury treatment, not maintenance in pools, represented a stress is clear from the observations that no fish survived in the high treatment pools and that the number of fish in the treated pools decreased but the number of fish increased in the controls. Differences in individual response might be linked to differences in metabolism as supported by our previous studies [18–20], especially those related to energy processing. Watt [39] has suggested that these differences in response might be strongly genotype dependent even for individual loci, if they have major roles in the flux of metabolites. The bioenergetic view suggested by Watt [39] predicts changes in pathway output with specific genetic changes and changes in fitness-related attributes.

Finally, we suggest that metabolic differences in *Gpi-2* genotypes (or closely linked loci) are expressed in stressed or fluctuating environments. This is likely a generalized response to stress rather than associated with pollutants per se. Thus, allozyme polymorphisms existing in populations with no history of contaminant stress can be subject to selection and provide the basis for adaptation to anthropogenic stress.

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