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RELATIONSHIPS BETWEEN MERCURY BODY CONCENTRATIONS, STANDARD METABOLIC RATE, AND BODY MASS IN EASTERN MOSQUITOFISH (*GAMBUSIA HOLBROOKI*) FROM THREE EXPERIMENTAL POPULATIONS

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Abstract—Eastern mosquitofish (*Gambusia holbrooki*) were sampled from three experimental populations (two Hg-exposed populations and one reference population) to determine whether transgenerational exposure (lifelong exposure of multiple generations) to Hg adversely affects standard metabolic rate (SMR). Mosquitofish subjected to lifelong Hg exposure accumulated significant concentrations of Hg in their tissues compared to fish from the reference population (mean: 3.89–4.13 vs 0.08 μHg/g wet mass, respectively). Less than 10% of the variability in Hg tissue concentrations could be explained by fish body mass, likely because of the short life span and/or dietary habits of this species. Despite the high body burdens of Hg in exposed fish, we found no significant difference in SMR among individuals from Hg-exposed or reference populations. Our findings contrast recent laboratory work describing elevated SMR in mosquitofish exposed to 100 μg/L dissolved inorganic Hg for 48 h. To account for contrasting results between studies, we hypothesize that acute exposure to dissolved inorganic Hg damages gill epithelium, resulting in increased metabolic rate, but that lifelong Hg exposure via trophic uptake of methyl mercury does not affect fish respiratory structures. Alternative hypotheses include the possibility that *G. holbrooki* is a species that can tolerate high body burdens of Hg or that more than four years of genetic isolation during Hg exposure (8–12 generations) resulted in selection for Hg-tolerant or -resistant individuals.

Keywords—Mercury Mosquitofish Respiration Standard metabolic rate Tolerance

INTRODUCTION

Exposure to metals often disturbs normal metabolic processes in fish. Such abnormalities emerge from various causes, including irritation of respiratory epithelium, changes in ventilation frequency, inefficient oxygen delivery to tissues, hyperactivity, and increased energy requirements to resist or repair toxicant-induced damage [1–3]. The net result of these disruptions is often an obligatory or compensatory increase in metabolic rate. If such increases in metabolism persist for long periods of time, they may ultimately translate to reduced growth, lifetime reproductive output, and survival in affected individuals [4]. In contrast, some metals are known to decrease metabolic rate in fishes [2]. Hypometabolism likely stems from damaged gill epithelium, neurotoxicity, or hypoactivity, three factors that can also ultimately affect growth, survival, and reproduction.

Recently, acute exposure to Hg was shown to increase oxygen consumption rate (VO_2) in eastern mosquitofish (*Gambusia holbrooki*). Mosquitofish exposed for 48 h to 100 μg/L $HgCl_2$ in the laboratory exhibited a 17% increase in VO_2 [5]. Under more ecologically realistic circumstances, fish would be exposed to Hg for longer periods of time, and exposure would occur mainly via trophic uptake of methyl-Hg [6–8]. Thus, the question remains as to whether environmentally relevant exposure to Hg would also result in altered respiration.

In the current study, we measured the VO_2 of mosquitofish from populations following transgenerational exposure (lifelong exposure of multiple generations) to Hg via ecologically meaningful exposure routes. We hypothesized that lifelong exposure to Hg would result in increased VO_2 similar to that previously observed in fish acutely exposed to dissolved inorganic Hg. We sampled individuals from two experimental populations that had been reared in Hg-contaminated artificial ponds for approximately four years and individuals from a third artificial pond that were not exposed to elevated Hg but were otherwise maintained under identical conditions. We compared the relationship between body size, Hg accumulation, and VO_2 among individuals from these three mosquitofish populations.

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METHODS

Fish exposure and sampling

Mosquitofish populations sampled for this study inhabited three 7,250-L artificial ponds constructed in January 1992 by Mulvey et al. [9]. Following construction, ponds were initially dosed to achieve three levels of dissolved Hg exposure: reference (<0.1 μg/L), 18 μg/L, and 42 μg/L. Mercury was added to the ponds once weekly as $HgCl_2$ until target water concentrations were achieved. Water column concentrations returned to background levels (<0.1 μg/L) within 72 h of dosing because of adsorption of Hg to particulate matter [9]. Ponds were stocked with mosquitofish in 1992 from an abandoned farm pond (Risher Pond, Savannah River Site, SC, USA) with no known history of Hg contamination [9]. The experimental ponds were allowed to dry in 1994, refilled with well water, and restocked with fish from Risher Pond [10]. Thus, fish from the three experimental populations were derived from a common parental stock in 1994 but were isolated from one another under different Hg exposure regimes until sampling in 1998 through 1999.

When the ponds were refilled in 1994, additional Hg was

not added; the sole source of Hg to the system was the contaminated sediments. After refilling, aquatic macrophyte, plankton, and insect communities became established. Thus, food webs similar to those expected in shallow warm-water ponds in the region were represented in the experimental ponds. Mosquitofish fed entirely on resources (e.g., algae, zooplankton, and insect larvae) present in the ponds. Measurement of dissolved Hg by a relatively insensitive method (cold vapor atomic fluorescence without preconcentration on gold traps) at the time the mosquitofish were collected (1998–1999) indicated that dissolved Hg concentrations in all three ponds was <50 ng/L. Measurements of total and methyl Hg in algae and benthos from the ponds suggest that Hg initially concentrated in the sediments has biomagnified in the aquatic food web. For example, measures of total Hg in algae samples collected from replicate experimental ponds ranged between 0.003 to 0.041 μg/g dry mass in a control pond versus 59.56 to 68.28 μg/g dry mass in a Hg-dosed pond (Jagoe and Unrine, University of Georgia, Aiken, SC, USA, unpublished data). Under conditions where dissolved Hg concentrations are low and concentrations in potential foods are relatively high, uptake via the gut is likely the predominant exposure pathway in both invertebrates [11] and fish [7]. Although mosquitofish may have been exposed to trace quantities of dissolved Hg in the Hg-dosed ponds, our measurements of Hg in the water and algae suggest that trophic exposure was the most important route of Hg uptake by fish in this study.

Fish were sampled from mesocosms from December 14, 1998, to March 4, 1999, (n = 21–39 individuals per population). We sampled one reference pond (population A), one pond initially spiked with 18 μg Hg/L (population B), and one pond initially spiked with 42 μg Hg/L (population C). Water chemistry during the collection period (dissolved oxygen, pH, conductance, and temperature) was collected using a Microsonde water quality microprobe (Hydrolab, Austin, TX, USA). Water quality parameters did not differ among experimental ponds (range: dissolved oxygen = 11–13 mg/L; specific conductance = 200–240 μs/cm; pH = 8.0–9.5 [12]). Fish were removed from mesocosms using dip nets and returned to the laboratory in water from their respective pond. Water was allowed to reach room temperature (20–22°C) overnight before gradually being exchanged with soft reconstituted freshwater [13] of the same temperature.

Respirometry

Fish respirometry procedures follow those previously described [3,5]. Briefly, fish were held for 2 d in aerated, soft reconstituted freshwater to ensure that they were postabsorptive prior to measures of respiration. Each postabsorptive fish was then placed in an individual 50-ml respiratory chamber containing soft reconstituted freshwater. Each respiratory chamber was connected to an individual channel on a closed-circuit, computer-controlled indirect respirometer (Micro-Oxymax, Columbus Instruments, Columbus, OH, USA). The computerized 20-chamber respirometer enables simultaneous measurements of oxygen consumption from 18 individuals as well as two additional chambers (a blank and a standard; 8.4-V zinc air medical battery, Duracell, Bethel, CT, USA) for quality control purposes. Respiratory chambers were placed within a dark environmental cabinet maintained at 22°C, and fish were allowed to acclimate in their chamber for at least 2 h prior to the first respiratory measurements. Oxygen consumption rates were determined at 2.1-h intervals for 24 h, with additional time allowed for oxygen refreshment following every two measurements. Eight oxygen consumption rates were obtained for each fish. Each respiratory session contained fish from at least two, but usually all three, of the experimental populations. Following each respiratory session, fish were removed from their chambers, blotted dry, weighed to the nearest milligram, and frozen (−70°C) for Hg analysis.

Mercury analysis

Fish were freeze-dried to a constant weight prior to analysis. Fish were digested in sets of 10 whole individuals, along with a blank and a standard reference material of known Hg content (National Research Council of Canada, Ottawa, ON). Samples were digested in closed Teflon® vessels within a microwave oven (CEM, Matthews, NC, USA) using ultra-high-purity nitric acid. Digestates were diluted to a constant volume with 1% BrCl in high-purity deionized water. Samples were analyzed within 24 h of digestion for total Hg using Brooks Rand Model 3 cold vapor atomic fluorescence spectrophotometer (Brooks Rand, Seattle, WA, USA) with high-purity N_2 as the carrier gas [14]. The instrument detection limit for Hg was 1.0 ng, and the limit of quantification was 3.0 ng. The instrument was calibrated daily using National Institute of Standards and Technology–traceable Hg standards (Gaithersburg, MD, USA). For quality assurance and control, replicates, spikes, and standards were included in the analyses and accounted for over 20% of the samples analyzed. All standard reference materials analyzed were within the certified range of Hg concentrations, and none of the blanks had detectable levels of Hg.

Data analysis

From the VO_2 data, we derived estimates of SMR using techniques previously described [3,15]. Inspection of the data obtained for individual fish revealed that some respiratory measures were elevated above the rest, likely because of periods of spontaneous activity during the respiratory session. Because SMR is an estimate of a postabsorptive ectotherm's metabolic rate at rest, such activity-influenced outliers must be removed from the data set to achieve a reliable estimate of SMR. Therefore, the upper 50% of VO_2 measures for each individual were removed from the data set. We used the mean of the remaining VO_2 measures (mean of the lower 50% of all measures) as our estimate of SMR for each individual. This method of data truncation produces robust estimates of SMR by removing variability in VO_2 values associated with activity bouts during the respiratory trial [3,15,16]. Data truncation resulted in a decrease in the variability (coefficient of variation = standard deviation × 100/mean) in oxygen consumption data for each fish from 19 to 14%, even though the coefficient of variation of the posttruncation data set is based on 50% fewer observations than the original data set.

Body mass, Hg concentration, and SMR were log transformed to meet assumptions of statistical models used. Whole-body concentrations of Hg were compared among the three populations using analysis of variance followed by Tukey's pairwise comparisons. The relationship between wet body mass and whole-body Hg concentration (μg/g wet mass) was examined among individuals within each population using regression analysis. To examine how body mass and Hg concentration influenced SMR, the relationship among body mass (g wet mass), Hg concentration (μg/g wet mass), and SMR was compared among the three populations using analysis of

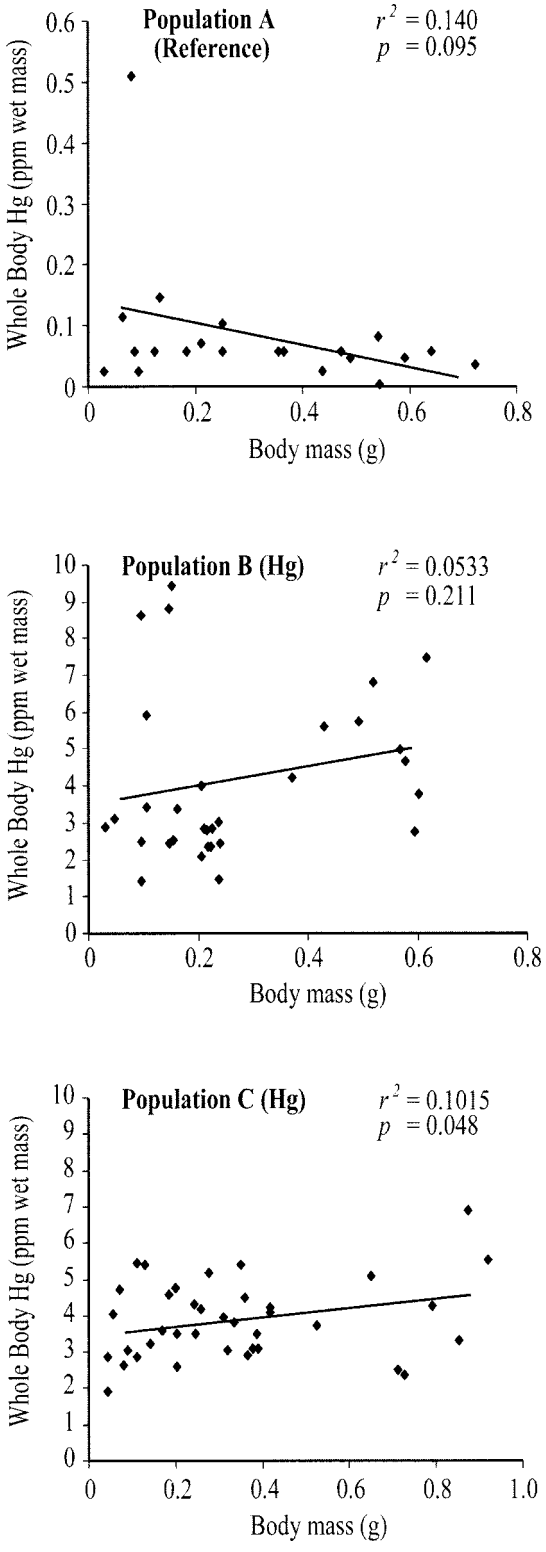

Fig. 1. The relationship between wet mass (g) and whole-body Hg concentration (μg/g wet mass) among mosquitofish (*Gambusia holbrooki*) collected from three experimental populations. Two of the populations (B and C) were exposed to Hg for approximately 8 to 12 generations (four years). The third population (A, reference) was derived from the same parental stock but was reared for 8 to 12 generations in a pond with no detectable Hg. Untransformed data are presented, but r^2 and p values represent results of linear regression on log-transformed data. Note that the scale on the y axis differs between reference and Hg-exposed populations because whole-body Hg concentrations were more than an order of magnitude lower in the reference population.

Table 1. Results of analysis of covariance investigating the relationship of body mass (g wet), whole-body Hg concentration (μg Hg/g wet mass), and standard metabolic rate (ml O_2/h) among eastern mosquitofish (*Gambusia holbrooki*) collected from a reference population and two Hg-exposed populations. All three parameters were log transformed prior to statistical analysis

Source	*df*	MS[a]	*F*	*p*
Population	2	0.01161	1.76	0.179
Mass	1	0.15163	22.93	<0.001
Mass × population	2	0.01431	2.16	0.122
Hg	1	0.00042	0.06	0.802
Hg × population	2	0.01809	2.74	0.071
Mass × Hg	1	0.01026	1.55	0.217
Mass × Hg × population	2	0.01820	2.75	0.070
Error	79	0.00612		

[a] MS = mean square.

covariance, with mass and Hg concentration used as covariates in the model.

RESULTS

Whole-body Hg concentrations differed significantly among the three populations sampled ($F_{2,88} = 635.37$, $p < 0.001$). Post hoc tests revealed that fish from the two Hg-dosed ponds had similar whole-body concentrations (μg Hg/g wet mass) of Hg (mean ± standard error, range; population B: 4.13 ± 0.37, 1.78–9.07; population C: 3.89 ± 0.16, 2.13–6.68). However, Hg concentrations in fish from both Hg-exposed populations were significantly higher than concentrations in fish from the reference population (population A: 0.08 ± 0.02, 0.01–0.51). Total mean moisture content of fish was 76.3 ± 0.21%. Whole-body Hg concentration was significantly related to fish body mass among fish within one of the Hg-treated ponds (population C) but not among fish in the other two ponds (Fig. 1).

Standard metabolic rate was influenced by body mass (Table 1 and Fig. 2), but not by Hg concentration (Table 1). As expected, SMR increased with body mass among fish within all three experimental populations. Although the slope of the line describing the relationship between body mass and oxygen consumption was reduced in population C compared to the two other populations, the slopes did not differ significantly from one another (as evidenced by the mass × population interaction term in the statistical model; Table 1).

DISCUSSION

Mosquitofish sampled from the two Hg-contaminated experimental ponds had high concentrations of Hg in their tissues. Mercury concentrations of fish from both exposed populations were more than 50 times higher than in mosquitofish from the control population and up to 25 times higher than background concentrations in natural fish populations of the South Carolina Coastal Plain, USA [17]. Moreover, Hg body burdens of individuals often exceeded threshold concentrations known to cause abnormalities in several fish species. Although fish species differ in sensitivity to Hg toxicity [18], whole-body concentrations of 5 to 10 μ g/g wet mass suggest probable toxic effects [7]. Examples of toxic effects observed at similar whole-body concentrations include hypoactivity, decreased feeding, diminished escape behavior, reduced growth, reduced reproductive output, and increased mortality [7,12,19,20].

Although Hg concentrations are often related to body mass in fishes [7], little of the variation in Hg concentration among

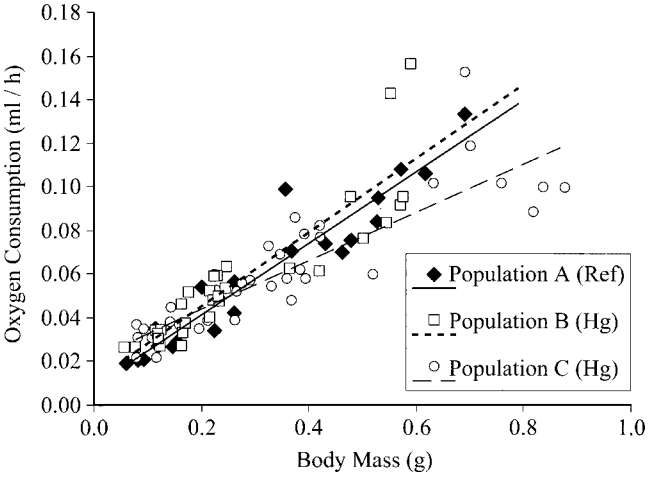

Fig. 2. The relationship between wet body mass (g) and standard metabolic rate (ml/h; estimated as oxygen consumption at 22°C) among mosquitofish (*Gambusia holbrooki*) from three experimental populations. Two of the populations (B and C) were exposed to Hg for approximately 8 to 12 generations. The third population (A; reference) was derived from the same parental stock but was reared for 8 to 12 generations in a pond with no detectable Hg.

mosquitofish in the two Hg-exposed populations was explained by body mass. The lack of a strong relationship between body mass and mercury may simply be attributable to the fact that mosquitofish have relatively short life spans and therefore do not live long enough to develop such a relationship. However, the lack of a body mass–mercury relationship may also relate to the dietary habits of mosquitofish. Mosquitofish reside primarily near the water surface, where they ingest a wide variety of food items, including algae, microcrustaceans, and insect larvae [21]. Whereas relationships between mass and Hg are sometimes weak in low-trophic-level fishes such as *G. holbrooki*, stronger relationships between mass and Hg concentrations are typically found in higher-trophic-level piscivorous fishes [17]. Further support for the influence of trophic position on the relationship between body mass and Hg concentration comes from studies of fish that exhibit ontogenetic shifts in diet from low- to high-trophic-level prey; species displaying such shifts exhibit stronger relationships between body mass and Hg accumulation later in life [17,22].

Despite the high concentrations of Hg accumulated by mosquitofish in the Hg-treated mesocosms, we found no difference in metabolic rate among individuals from the three populations. This finding contrasts the work of Tatara et al. [5], who found that acute exposure to dissolved inorganic Hg increased metabolic rate in mosquitofish from the same source population. We hypothesize that the most probable explanation for this discrepancy relates to differences in Hg exposure conditions between the two studies. Exposure to dissolved inorganic Hg disrupts gill epithelium, potentially affecting gas exchange and permeability of cell membranes to cations [18,23,24]. Such disruptions may result in compensatory changes in ventilation frequency, increased energy demands, or altered gas exchange efficiency, possibly resulting in the increase in metabolic rate observed by Tatara et al. [5]. In contrast, most Hg accumulation by mosquitofish in the present study probably occurred via intestinal absorption from dietary sources. Gill damage and subsequent increases in SMR might not occur following dietary exposure to Hg.

Besides the differences in Hg exposure conditions between the current study and Tatara et al. [5], another explanation for the absence of increased SMR in our study may relate to the general tolerance of mosquitofish to chronic environmental contamination. Mosquitofish have repeatedly been shown to tolerate high levels of metal exposure [9,10,12,25–27]. For example, a recent study by Staub [27] demonstrated that mosquitofish sampled from a population entrained within a coal ash settling basin for more than 25 years exhibited normal SMR compared to unexposed conspecifics despite high body burdens of numerous trace elements, including As, Se, Cu, Cr, and Cd. The general tolerance of mosquitofish to metals is supported by additional studies; reduced growth, altered swimming performance, reduced survival, reproductive impairment, and population declines occur under conditions associated with trace element contamination in various fish species [3,16,26,28], but mosquitofish populations persist in the same contaminated environments [25–27].

We cannot eliminate the possibility that isolation of mosquitofish populations in the experimental ponds led to selection of traits for Hg resistance or tolerance and subsequent attenuation of effects on metabolic rate. Assuming that two to three generations elapse per year [29], approximately 8 to 12 generations of mosquitofish had elapsed in the isolated experimental populations prior to this study. Previous work indicates that after two years of isolation, mosquitofish from the Hg-contaminated experimental ponds exhibited differences in allele (glucosephosphate isomerase-2; *Gpi-2*) frequencies, suggesting population changes might be occurring in response to Hg exposure [10]. The differences in *Gpi-2* allele frequencies persisted in Hg-contaminated ponds after four years of isolation [12]. However, changes in *Gpi-2* allele frequencies did not appear to be associated with resistance to Hg-induced changes in SMR [5] or reproductive parameters [12]. Other experimental evidence suggests that adaptation is not necessarily requisite for persistence of mosquitofish populations under heavily contaminated conditions. For example, Staub [27] found that feeding trace element–contaminated food to mosquitofish from a reference population for 78 d had no effect on survival, growth, or SMR. These findings, coupled with Staub's [27] fieldwork (discussed previously), suggests that mosquitofish are generally tolerant of, rather than adapted to, the contaminated conditions he studied. The issue of inherent tolerance versus adaptation to Hg in mosquitofish can be addressed by future experiments that compare responses (e.g., SMR and growth) of mosquitofish from a reference site experimentally exposed to dietary Hg to responses of mosquitofish from populations exposed to dietary Hg transgenerationally.

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