

Size-Dependence of Mercury (II) Accumulation Kinetics in the Mosquitofish, *Gambusia affinis* (Baird and Girard)

Michael C. Newman and Danette K. Doubet

Savannah River Ecology Laboratory, P.O. Drawer E, Aiken, South Carolina 29801, USA

Abstract. Size-dependence of mercury (II) accumulation from water by the mosquitofish, *Gambusia affinis* was assessed under controlled laboratory conditions. Uptake rates were higher for smaller fish than for larger fish. Mean (\pm S.D.) uptake rate for mosquitofish exposed to 0.24 $\mu\text{g/L}$ of Hg was $0.32 \pm 0.15 \mu\text{g/g dry wt/day}$. Uptake rate constants were similar for the Hg (II) and Hg^0 as reported elsewhere. Both inorganic species (Hg (II) and Hg^0) were accumulated faster than methylmercury. Elimination rate constants averaged 0.53 ± 0.14 per day (mean \pm 1 S.D.). No significant size effects on elimination rate constants were detected. Elimination constants were similar to those reported elsewhere for Hg^0 elimination but larger than those for methylmercury elimination.

Animal size can influence pollutant accumulation by aquatic biota. Numerous field studies have demonstrated that the relation between trace element body burden and individual size can be readily defined by a power model (Boyden 1974, 1977; Cossa *et al.* 1980; Williamson 1980; Watling *et al.* 1981; Strong and Luoma 1981; Newman and McIntosh 1983). The exponents or b-values for such empirical models tend toward either 0.75 or 1.00 for many species/trace element combinations but range from less than 1 to greater than 2 (Boyden 1977). Size-specific metabolic rate has been assumed to be the mechanism controlling relations with b-values of approximately 0.75 (Baker and Dunaway 1969; Pulliam *et al.* 1969; Boyden 1974). However, Fagerstrom (1977) demonstrated that, under equilibrium conditions, body burden versus body weight relations with b-values near unity are more indicative of linkage to metabolic rate. To date, the importance of size-specific metabolic rate in determining

size-dependence of trace element accumulation remains ambiguous.

Although mechanisms controlling size-dependence of accumulation remain poorly defined in most studies, a clearer understanding of the relationship between trace element accumulation kinetics and organism size is essential for development of complex models linked to bioenergetics (Norstrom *et al.* 1976). To this end, Newman and Mitz (1988) defined the relations between mosquitofish size and the variables defining elimination and uptake of Zn. The slow turnover relative to fish life expectancy imparted a nonequilibrium nature to Zn accumulation in the mosquitofish. In contrast to Zn accumulation, the accumulation of inorganic Hg, another group IIb metal, is rapid in many fish species (McKone *et al.* 1971; Burrows and Krenkel 1973; Pentreath 1976a, 1976b), including the mosquitofish (Schoper 1974; Boudou *et al.* 1979). Therefore, Hg accumulation in mosquitofish will not have the nonequilibrium characteristics of Zn accumulation in mosquitofish. The results discussed herein describe the size-specific relations for Hg (II) uptake and elimination for the model organism, *Gambusia affinis*. The rates and rate constants were compared to those for methylmercury and Hg^0 accumulation. These results will be used to develop a complex accumulation model incorporating allometric and bioenergetic characteristics of this species.

The mosquitofish was selected as the model species for several reasons. It is small, hardy and easily reared in the laboratory. It is common and widespread in warm regions of the world. Its original distribution in the southeastern United States has been broadened to the widest of any freshwater fish (Krumholz 1948), chiefly because of its value in controlling mosquitos. Finally, an abundance of studies focusing on mosquitofish ecology (Krum-

holz 1948; Bence and Murdoch 1986), bioenergetics (Cech *et al.* 1980, 1984; Wurtsbaugh and Cech 1983; Bence and Murdoch 1986), population dynamics (Brown 1985; Kennedy *et al.* 1986), toxicant bioaccumulation (Hannerz 1968; Schoper 1974; Huckabee *et al.* 1975; Shin and Krenkel 1976; Williams and Giesy 1978; Boudou *et al.* 1979; Saiki 1987; Newman and Mitz 1988) and toxicity (Wallen *et al.* 1957; Boyd and Ferguson 1964a, 1964b) exists; thus, providing a rich body of background information.

Materials and Methods

Mosquitofish

Various sized mosquitofish were collected from Risher Pond on the Savannah River Plant (Barnwell County, South Carolina) in late June 1987. As atypical elimination has been noted for gravid mosquitofish (Newman and Mitz 1988), gravid mosquitofish were excluded from the experiment. Male fish were not used in the experiment. Fish were maintained in 3-L glass aquaria at 20°C in a GCA Precision incubator. A photoperiod of 14 hr light and 10 hr dark was maintained. During the 10 day acclimation period, fish were fed commercial (Blue Ribbon Tubifex[®]) freeze-dried tubificid worms daily. Mean (S.D.) concentration for 3 samples of this material was $0.09 \pm 0.01 \mu\text{g Hg/g}$ dry wt. Holding and experimental waters were reconstituted, very soft freshwater (RVSWF) (EPA 1978). Calcium sulfate (0.250 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.150 g), NaHCO_3 (0.200 g) and KCl (0.025 g) were added to 50 L of deionized water to produce the RVSWF.

Experimental Exposure

Mercury (II) accumulation in fish of various sizes was measured using the radionuclide ^{203}Hg (Exposure Treatment). A second group of fish (Nonexposure Treatment) that was not exposed to ^{203}Hg was used to define background conditions during accumulation. Fish growth, food uptake and water quality were measured using the nonexposure treatment. Nonexposure and ^{203}Hg -exposure fish were held at 20°C in individual plexiglas chambers equipped with Nytex mesh bottoms as described in Newman and Mitz (1988). All plexiglas chambers were placed within two 25 L plexiglas tanks. The volume of each chamber was scaled to fish size. The chambers ranged in size from 1.5 cm W \times 5 cm L \times 14 cm H to 26 cm L \times 10.5 cm W \times 14 cm H. This arrangement allowed the water within each 25-L tank to circulate freely between all individual chambers. The nonexposure tank received a nominal concentration of $0.250 \mu\text{g Hg/L}$ (as HgCl_2) and the ^{203}Hg exposure tank received $0.250 \mu\text{g Hg/L}$ and $0.10 \mu\text{Ci}^{203}\text{Hg/L}$.

Individual fish were counted for 6 min each with a Beckman Gamma 8000 gamma counter after 0, 1, 2, 3, 4 and 6 days of exposure. The 279 KeV gamma photon was measured, using a window of 100 to 300 KeV. Geometry changes associated with counting live fish were insignificant (Newman and Mitz 1988). After removal from the individual compartment, a fish was immersed consecutively in 5 volumes of RVSWF. The rinse waters

Table 1. Water quality in experimental aquaria

Variable	Mean	Standard deviation	N ^a
Temperature (°C)	19.7	0.3	9
Dissolved oxygen (mg O ₂ /L)	6.5	1.6	5
Percent oxygen saturation (%)	71	18	5
pH ^b	6.05	6.00-6.40	5
Ca (mg/L)	1.3	0.3	5
Mg (mg/L)	0.34	0.03	5
Na (mg/L)	1.3	0.1	5
Total Alkalinity (mg/L as CaCO ₃)	6.6	1.6	5
Cl (mg/L)	0.5	0.2	5
SO ₄ (mg SO ₄ /L)	5.2	0.2	5
Dissolved Hg (μg/L)	0.24	0.07	5
²⁰³ Hg activity (μCi/L) ^c	0.8 ^c	0.6	11

^a Number of samples analyzed

^b Median and range

^c Activity measured in exposure tank

were counted after removal of the fish. No activity was measured after the second rinse; therefore, 3 rinses of 250 ml RVSWF was considered adequate to remove any residual exposure water on the fish prior to counting. An additional fish was also immersed in exposure water for 10 min, rinsed and counted to ensure that activity was due to uptake primarily, not immediate adsorption by body surface mucus (McKone *et al.* 1971; Schoper 1974). No rapid adsorption to body mucus was apparent. Schoper (1974) found 13 to 19% of Hg²⁺ associated with mosquitofish exposed to $0.100 \mu\text{g/ml}$ was adsorbed to body surface mucus.

Water Quality

One half of the water was removed daily from each tank and replaced with $^{203}\text{Hg}/\text{Hg}$ or Hg spiked RVSWF. Water samples were taken before and several hours after replacement to assay the ^{203}Hg activity in the exposure tank. Fifteen ml of water were counted for activity estimation.

Water quality was determined throughout the exposure period (Table 1). Temperature was taken daily from the control tank just prior to feeding. All other chemistries were taken every other day from the nonexposure tank before water change. Dissolved oxygen was determined with the sodium azide modification of the Winkler method (APHA 1975). Total alkalinity was quantified by potentiometric titration (APHA 1975). Chloride and sulfate concentrations were determined with a Dionex[®] 4000i ion chromatograph (HPIC AS4A column and 4.0 mM Na₂CO₃/1.5 mM NaHCO₃ eluant). Sodium, K, Mg and Ca were determined with a Hitachi 180-80 flame atomic absorption spectrophotometer equipped with Zeeman background correction. Lanthanum chloride was added to samples prior to Ca analysis. Ionic balance estimations were performed using the MAQA program (Hill 1984). Dissolved Hg samples were filtered ($0.45 \mu\text{m}$ HAWP04700 filter) and preserved with Ultrex nitric acid. After a hot digestion (APHA 1975), all samples were analyzed with a Perkin-Elmer 50A mercury analyzer.

Feeding and Growth

Two potentially complicating factors, fish growth and uptake from food, were assessed during Hg accumulation experiments. For reasons of radiation safety, feeding and growth rates were monitored in the nonexposure tank. Growth could produce a diluting effect for the assimilated Hg as the Hg would be distributed in increasing amounts of tissue as the fish grew. Growth was assessed by weighing nonexposure fish before and after the six days of accumulation. Also, Hg adsorbed to food during the 4 hr of feeding could become a second source of Hg to the fish. Tubifex worm rations were weighed prior to presentation to each fish. The amount of food presented to each fish was proportional to fish size. The animals were allowed to feed undisturbed for 4 hr. Significant amounts of food were present at the end of the feeding periods for all fish indicating *ad libitum* feeding during this period. All uneaten food was removed, freeze-dried and reweighed to determine the amount of food consumed by each fish. A range of various sized food samples were also exposed to 0.10 $\mu\text{Ci}^{203}\text{Hg}/\text{L}$ and 0.250 $\mu\text{g Hg}/\text{L}$ at 20°C for 1, 2, 3 and 4 hr to assess the degree of adsorption of Hg to the food during the period of feeding. The range of sizes for the exposed food reflected the range presented to the fish during the actual accumulation experiment.

A significant, size-dependent feeding rate relation was determined by least-squares regression methods to fit a power model (Eq. 1). The Statistical Analysis System (SAS) (SAS Institute 1985) computer package procedure GLM was used to fit a linear model to the logarithm of fish weight versus the logarithm of consumption rate. The bias associated with results from regression of log transformed data was corrected using the methods of Beauchamp and Olson (1973).

$$C = aW^b \quad (1)$$

where C = consumption rate (g dry wt/fish/day),
W = fish weight (g dry wt), and
a, b = constants derived from the regression model.

Accumulation Kinetics

The change in Hg concentration within each fish was estimated using the specific activity calculated from the water source and the gamma activity in the fish. These data were fitted to the following model using nonlinear regression methods in the SAS procedure NLIN (Secant method) (SAS Institute 1985).

$$C_t = (k_u/k_e)(1 - e^{-(k_e + \lambda)t}) \quad (2)$$

where C_t = concentration at time, t ($\mu\text{g}/\text{g}$ dry wt),
 k_u = the uptake rate ($\mu\text{g}/\text{g}$ dry wt/day),
 k_e = the elimination rate constant (1/day),
 λ = decay rate constant for ^{203}Hg (1/day), and
t = time (day).

With the units given above, k_u/k_e is equal to the concentration at equilibrium (C_{eq}).

Two of the 21 fish exposed to the ^{203}Hg did not conform to the model described by Eq. 2. Each had a distinct lag that imparted a sigmoidal shape to the accumulation curves. Within the constraints of the experimental design, a specific mechanism such as saturation of the elimination mechanism (Spacie and Hame-link 1985), time lag before clearance as facilitated by internal conversion (Matis 1972), compartmental heterogeneity (Matis *et al.* 1983) or age-dependence of atom transition from a compart-

ment (Hughes and Matis 1984) could not be identified for incorporation into an accumulation model. Time dependence of uptake from one of two sources such as described by Huckabee *et al.* (1975) was rejected as a possible mechanism for these atypical accumulation curves. As suggested by Dr. I. L. Brisbin, Jr. (pers. comm.) and the thesis of Peters (1986), a reparameterized Richards' equation (Brisbin *et al.* 1986) was used for estimating accumulation parameters for the atypical accumulation curves. Briefly, the model was the following:

$$C_t = [C_{eq}^{1-M}(1 - e^{-(k_e + \lambda)(t-M)})]^{1/(1-M)} \quad (3)$$

where C_{eq} = the equilibrium concentration ($\mu\text{g}/\text{g}$ dry wt),
M = a shape parameter, and
 C_t, k_e, λ, t = same as defined in Eq. 2.

Again, with the units used in this equation, C_{eq} is equal to the uptake rate divided by the elimination rate constant. When M is equal to 0, Eq. 3 reduces to Eq. 2. A family of curves including the logistic or autocatalytic (M = 2), Gompertz (M = 1), Van Bertalanffy's (M = 0.67) and monomolecular (Eq. 2, M = 0) models can be generated using this equation (Richards 1959). Accumulation parameters can then be estimated for the fish displaying typical and atypical accumulation curves.

Results

Water Quality

Table 1 summarizes the water quality within the tanks during the 6 days of exposure. Oxygen concentrations were approximately 71% of theoretical saturation. The RVSWF was slightly acidic (6.05) and temperatures within the tanks were approximately 20°C. Acceptable ionic balance (2.6% deviation from perfect balance) was evident for the major ion analyses.

Growth and Feeding Rate Estimates

Wet and dry weights for fish in the nonexposed group were used to evaluate a dry:wet weight ratio for all fish. Nineteen paired observations produced a least-squares regression model (total df = 18, $r^2 = 0.98$) indicating that wet weight multiplied by 0.26 yields an accurate measure of fish dry weight. This value was similar to that derived by Williams and Giesy (1978) for mosquitofish from Risher Pond (0.21). There was no apparent deviation from linearity over the range of fish measured.

Dry weights for fish exposed to $^{203}\text{Hg}/\text{Hg}$ ranged from 0.0073 to 0.3908 g, more than a fifty-fold difference in weight between individuals. Under the holding conditions and accuracy of weighing methods, there was a minor increase in fish weight during the exposure period. No clear trend was noted between percentage weight increase and fish size. Wurtsbaugh and Cech (1983) report significant

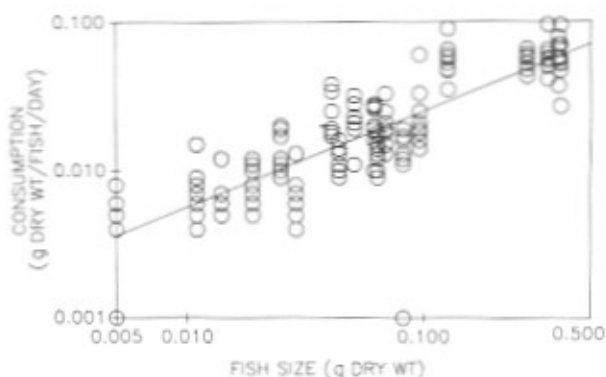


Fig. 1. Amount of food consumed per day by various-sized, nonexposure mosquitofish

size dependent growth rates for mosquitofish held at 10 to 35°C and fed live food. Under a restricted feeding regime (20% of body weight per day at 20°C), mosquitofish growth rate was decreased threefold to approximately 2% per day for juveniles (Wurtsbaugh and Cech 1983). Mosquitofish in the present experiment were consuming approximately 25% of their body weight per day. However, at the end of 6 days, there was no significant growth ($4 \pm 7\%$ S.D. of initial dry weight). This slow growth (0.7% per day) indicated that the feeding schedule and freeze-dried food were suboptimal for growth. The negligible and apparently size-independent rate of growth under these conditions was not incorporated into the accumulation model.

Feeding rate for the control animals was clearly size-dependent (Figure 1). The least-squares regression of log food consumption (g dry wt/fish) versus log fish weight (g dry wt) produced a slope of 0.65 ± 0.03 (S.E.) and intercept of -0.95 ± 0.04 (S.E.). The r^2 for this model was 0.71 (total df = 151). A uniform distribution of residuals from the model was noted when residuals were plotted against fish weight. Therefore, the power model was judged adequate for these data. Food consumption for the exposure fish was estimated using this relation.

Estimated Uptake of Hg from Food

Food cubes of 0.01, 0.03 and 0.25 g dry weight rapidly adsorbed $^{203}\text{Hg}(\text{II})$ to a maximum activity of $0.7 \mu\text{Ci/g}$ dry wt within 2 hr of exposure to spiked RVSFW. Using the size-specific, consumption rate relation and fish dry weight estimates, a daily intake rate for Hg(II) in food was estimated for each exposure fish. However, prior to approximating intake from food, the percentage of Hg retained

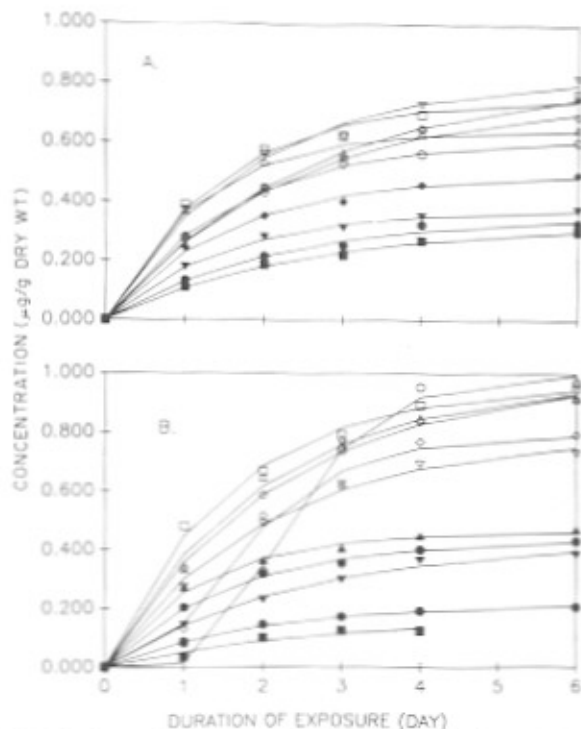


Fig. 2. Accumulation curves for 21 various-sized mosquitofish. Final fish dry weights (g) were the following (top to bottom at six day). Figure 2A: 0.0679 (∇), 0.0949 (\square), 0.0836 (Δ), 0.0826 (\circ), 0.2464 (Δ), 0.0784 (\circ), 0.3908 (\blacklozenge), 0.2795 (\blacktriangledown), 0.3030 (\bullet), and 0.2748 (\blacksquare). Fig. 2B: 0.0074 (\circ), 0.0413 (\square), 0.0242 (\circ), 0.0239 (\blacktriangledown), 0.0342 (\circ), 0.0160 (∇), 0.1286 (\blacktriangle), 0.0813 (\bullet), 0.0894 (\blacktriangledown), 0.0876 (\bullet), and 0.1218 (\blacksquare). The last fish (0.1218 g dry wt) was damaged in transfer during counting and died soon after day 4 of exposure. Two fish (Figure 2B: 0.0074 and 0.0342 g dry wt) displayed atypical, sigmoidal accumulation curves

within the fish after food intake had to be considered. Pentreath (1976b) measured a retention percentage of inorganic Hg from food in the plaice of 10%. Even assuming an extreme retention percentage of 100% for the mosquitofish, the percentage of the total Hg body burden after 6 days resulting from food consumption was insignificant (Mean \pm S.D.: $1.1 \pm 0.7\%$, $n = 21$). Therefore, $^{203}\text{Hg}(\text{II})$ uptake from food as a second source of ^{203}Hg to exposure fish was deemed insignificant and not incorporated into the accumulation models.

Mercury Accumulation from Water

With the exception of two fish as noted earlier, the model described by Eq. 2 fit the accumulation data for mosquitofish (Figure 2). When the Richards' model (Eq. 3) was employed for the atypical fish, the respective residual sums of squares dropped

Feeding and Growth

Two potentially complicating factors, fish growth and uptake from food, were assessed during Hg accumulation experiments. For reasons of radiation safety, feeding and growth rates were monitored in the nonexposure tank. Growth could produce a diluting effect for the assimilated Hg as the Hg would be distributed in increasing amounts of tissue as the fish grew. Growth was assessed by weighing nonexposure fish before and after the six days of accumulation. Also, Hg adsorbed to food during the 4 hr of feeding could become a second source of Hg to the fish. Tubifex worm rations were weighed prior to presentation to each fish. The amount of food presented to each fish was proportional to fish size. The animals were allowed to feed undisturbed for 4 hr. Significant amounts of food were present at the end of the feeding periods for all fish indicating *ad libitum* feeding during this period. All uneaten food was removed, freeze-dried and reweighed to determine the amount of food consumed by each fish. A range of various sized food samples were also exposed to 0.10 $\mu\text{Ci}^{203}\text{Hg/L}$ and 0.250 $\mu\text{g Hg/L}$ at 20°C for 1, 2, 3 and 4 hr to assess the degree of adsorption of Hg to the food during the period of feeding. The range of sizes for the exposed food reflected the range presented to the fish during the actual accumulation experiment.

A significant, size-dependent feeding rate relation was determined by least-squares regression methods to fit a power model (Eq. 1). The Statistical Analysis System (SAS) (SAS Institute 1985) computer package procedure GLM was used to fit a linear model to the logarithm of fish weight versus the logarithm of consumption rate. The bias associated with results from regression of log transformed data was corrected using the methods of Beauchamp and Olson (1973).

$$C = aW^b \quad (1)$$

where C = consumption rate (g dry wt/fish/day),
W = fish weight (g dry wt), and
a, b = constants derived from the regression model.

Accumulation Kinetics

The change in Hg concentration within each fish was estimated using the specific activity calculated from the water source and the gamma activity in the fish. These data were fitted to the following model using nonlinear regression methods in the SAS procedure NLIN (Secant method) (SAS Institute 1985).

$$C_t = (k_u/k_e)(1 - e^{-(k_e + \lambda)t}) \quad (2)$$

where C_t = concentration at time, t ($\mu\text{g/g}$ dry wt),
 k_u = the uptake rate ($\mu\text{g/g}$ dry wt/day),
 k_e = the elimination rate constant (1/day),
 λ = decay rate constant for ^{203}Hg (1/day), and
t = time (day).

With the units given above, k_u/k_e is equal to the concentration at equilibrium (C_{eq}).

Two of the 21 fish exposed to the ^{203}Hg did not conform to the model described by Eq. 2. Each had a distinct lag that imparted a sigmoidal shape to the accumulation curves. Within the constraints of the experimental design, a specific mechanism such as saturation of the elimination mechanism (Spacie and Hamelink 1985), time lag before clearance as facilitated by internal conversion (Matis 1972), compartmental heterogeneity (Matis *et al.* 1983) or age-dependence of atom transition from a compart-

ment (Hughes and Matis 1984) could not be identified for incorporation into an accumulation model. Time dependence of uptake from one of two sources such as described by Huckabee *et al.* (1975) was rejected as a possible mechanism for these atypical accumulation curves. As suggested by Dr. I. L. Brisbin, Jr. (pers. comm.) and the thesis of Peters (1986), a reparameterized Richards' equation (Brisbin *et al.* 1986) was used for estimating accumulation parameters for the atypical accumulation curves. Briefly, the model was the following:

$$C_t = [C_{eq}^{1-M}(1 - e^{-(k_e + \lambda)(1-M)t})]^{1/(1-M)} \quad (3)$$

where C_{eq} = the equilibrium concentration ($\mu\text{g/g}$ dry wt),
M = a shape parameter, and
 C_t , k_e , λ , t = same as defined in Eq. 2.

Again, with the units used in this equation, C_{eq} is equal to the uptake rate divided by the elimination rate constant. When M is equal to 0, Eq. 3 reduces to Eq. 2. A family of curves including the logistic or autocatalytic (M = 2), Gompertz (M = 1), Van Bertalanffy's (M = 0.67) and monomolecular (Eq. 2, M = 0) models can be generated using this equation (Richards 1959). Accumulation parameters can then be estimated for the fish displaying typical and atypical accumulation curves.

Results

Water Quality

Table 1 summarizes the water quality within the tanks during the 6 days of exposure. Oxygen concentrations were approximately 71% of theoretical saturation. The RVSWF was slightly acidic (6.05) and temperatures within the tanks were approximately 20°C. Acceptable ionic balance (2.6% deviation from perfect balance) was evident for the major ion analyses.

Growth and Feeding Rate Estimates

Wet and dry weights for fish in the nonexposed group were used to evaluate a dry:wet weight ratio for all fish. Nineteen paired observations produced a least-squares regression model (total df = 18, $r^2 = 0.98$) indicating that wet weight multiplied by 0.26 yields an accurate measure of fish dry weight. This value was similar to that derived by Williams and Giesy (1978) for mosquitofish from Risher Pond (0.21). There was no apparent deviation from linearity over the range of fish measured.

Dry weights for fish exposed to ^{203}Hg ranged from 0.0073 to 0.3908 g, more than a fifty-fold difference in weight between individuals. Under the holding conditions and accuracy of weighing methods, there was a minor increase in fish weight during the exposure period. No clear trend was noted between percentage weight increase and fish size. Wurtsbaugh and Cech (1983) report significant

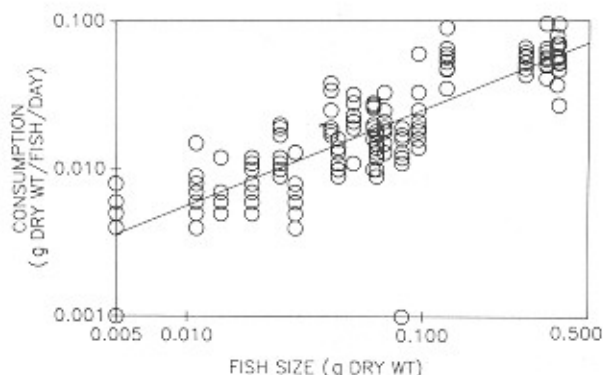


Fig. 1. Amount of food consumed per day by various-sized, nonexposure mosquitofish

size dependent growth rates for mosquitofish held at 10 to 35°C and fed live food. Under a restricted feeding regime (20% of body weight per day at 20°C), mosquitofish growth rate was decreased threefold to approximately 2% per day for juveniles (Wurtsbaugh and Cech 1983). Mosquitofish in the present experiment were consuming approximately 25% of their body weight per day. However, at the end of 6 days, there was no significant growth ($4 \pm 7\%$ S.D of initial dry weight). This slow growth (0.7% per day) indicated that the feeding schedule and freeze-dried food were suboptimal for growth. The negligible and apparently size-independent rate of growth under these conditions was not incorporated into the accumulation model.

Feeding rate for the control animals was clearly size-dependent (Figure 1). The least-squares regression of log food consumption (g dry wt/fish) versus log fish weight (g dry wt) produced a slope of 0.65 ± 0.03 (S.E.) and intercept of -0.95 ± 0.04 (S.E.). The r^2 for this model was 0.71 (total df = 151). A uniform distribution of residuals from the model was noted when residuals were plotted against fish weight. Therefore, the power model was judged adequate for these data. Food consumption for the exposure fish was estimated using this relation.

Estimated Uptake of Hg from Food

Food cubes of 0.01, 0.03 and 0.25 g dry weight rapidly adsorbed $^{203}\text{Hg}(\text{II})$ to a maximum activity of $0.7 \mu\text{Ci/g}$ dry wt within 2 hr of exposure to spiked RVSW. Using the size-specific, consumption rate relation and fish dry weight estimates, a daily intake rate for Hg(II) in food was estimated for each exposure fish. However, prior to approximating intake from food, the percentage of Hg retained

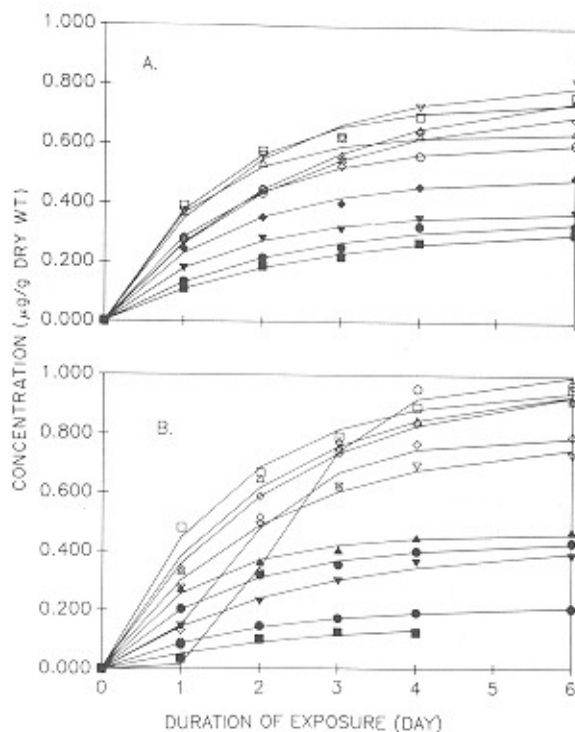


Fig. 2. Accumulation curves for 21 various-sized mosquitofish. Final fish dry weights (g) were the following (top to bottom at six days). Figure 2A: 0.0679 (∇), 0.0949 (\square), 0.0836 (Δ), 0.0826 (\diamond), 0.2464 (\triangle), 0.0784 (\circ), 0.3908 (\blacklozenge), 0.2795 (\blacktriangledown), 0.3030 (\bullet), and 0.2748 (\blacksquare). Fig. 2B: 0.0074 (\circ), 0.0413 (\square), 0.0242 (\triangle), 0.0239 (\blacktriangledown), 0.0342 (\blacklozenge), 0.0160 (∇), 0.1286 (\blacktriangle), 0.0813 (\bullet), 0.0894 (\blacktriangledown), 0.0876 (\blacklozenge), and 0.1218 (\blacksquare). The last fish (0.1218 g dry wt) was damaged in transfer during counting and died soon after day 4 of exposure. Two fish (Figure 2B: 0.0074 and 0.0342g dry wt) displayed atypical, sigmoidal accumulation curves

within the fish after food intake had to be considered. Pentreath (1976b) measured a retention percentage of inorganic Hg from food in the plaice of 10%. Even assuming an extreme retention percentage of 100% for the mosquitofish, the percentage of the total Hg body burden after 6 days resulting from food consumption was insignificant (Mean \pm S.D.: $1.1 \pm 0.7\%$, $n = 21$). Therefore, $^{203}\text{Hg}(\text{II})$ uptake from food as a second source of ^{203}Hg to exposure fish was deemed insignificant and not incorporated into the accumulation models.

Mercury Accumulation from Water

With the exception of two fish as noted earlier, the model described by Eq. 2 fit the accumulation data for mosquitofish (Figure 2). When the Richards' model (Eq. 3) was employed for the atypical fish, the respective residual sums of squares dropped

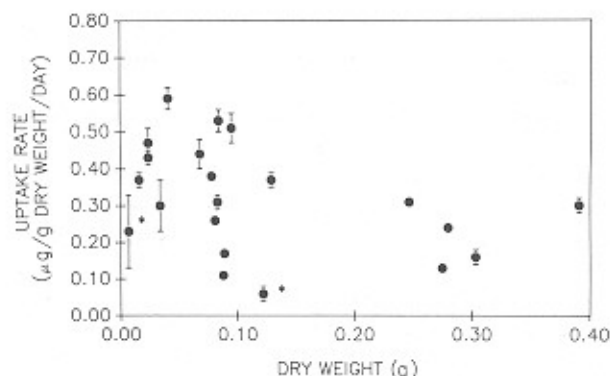


Fig. 3. Uptake rate (K_u) for inorganic mercury uptake by mosquitofish. Error bars denote the standard error of the estimate; several error bars were sufficiently small as to be contained within the symbols denoting estimate points. The estimates for the two, atypical mosquitofish are indicated by the symbol, *. Note the relatively wide standard errors associated with the estimates for these two fish. The estimate for the fish that died prior to day 6 is indicated by the symbol ‡

from 0.0273 and 0.0829 to 0.0054 and 0.0037. The pattern of the residuals showed no trends after fitting the Richards' model indicating acceptable model fit to these data.

Uptake of Hg (II) (Figure 3) was rapid. Uptake rates for the 19 typical fish exposed to 0.24 μg Hg/L averaged 0.32 ± 0.15 S.D. μg Hg/g dry wt/day. The mean and standard deviation for all 21 fish including values derived using the Richards' equation for the two atypical fish were 0.31 ± 0.14 μg Hg/g dry wt/day ($n = 21$). Kendall rank correlation analysis indicated a significant effect of fish size on uptake rate of Hg (II) (tau-b coefficient = 0.3941, $P = 0.0189$, $n = 19$). Generally, smaller fish had higher uptake rates for Hg (II) than larger fish. However, wide variation between individuals independent of fish size precluded development of a viable statistical model for size versus uptake rate.

Elimination of Hg was also determined to be rapid for all fish (Figure 4). Elimination rate constants estimated using Eq. 2 for the 19 typical fish averaged 0.53 per day with a standard deviation of 0.14. When the sigmoidal model was employed for the two additional fish, the overall mean and standard deviation for the 21 fish were 0.49 ± 0.18 per day. The biological half-life, t_{50} was estimated to be 1.4 days and the time to 95% equilibrium concentration ($t_{95} = -(\ln 0.05/k_e)$) was approximately 6 days. Using Kendall rank correlation methods (SAS procedure CORR with the Kendall's tau-b option), no significant concordance nor discordance was noted between fish size and elimination rate constant (tau-b coefficient = -0.0118, $P = 0.9441$, $n = 19$).

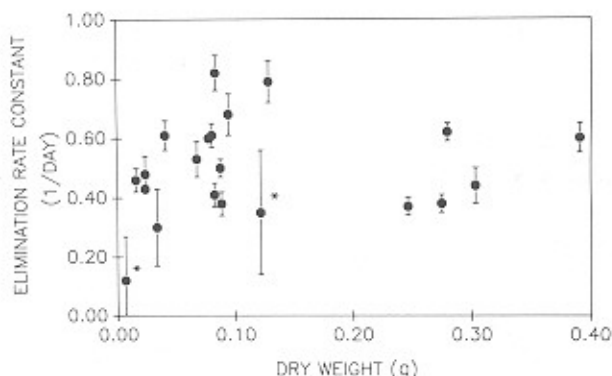


Fig. 4. Elimination rate constant (K_e) for inorganic mercury elimination by mosquitofish. Error bars denote the standard error of the estimate. Estimates for the two, atypical mosquitofish and indicated by the symbol, *. The estimate for the fish that died prior to day 6 is indicated by the symbol, ‡. Note the relatively wide standard errors for the estimates associated with the atypical fish

Discussion

Mercury Species Comparison Data

Using the model described by Eq. 2 and the results of Schoper (1974) and Huckabee *et al.* (1975), rates and rate constants were derived for methylmercury and Hg^0 accumulation by mosquitofish held at approximately 20°C. Although Schoper (1974) used higher concentrations (0.10 $\mu\text{g}/\text{ml}$) and temperatures (23°C) than the present study and Huckabee *et al.* (1975), Schoper compared Hg^0 and mercuric chloride accumulation by mosquitofish under identical conditions. The internal consistency with her work allows indirect comparison to Huckabee *et al.* (1975) and the present study.

Uptake

The present study, Schoper (1974) and Huckabee *et al.* (1975) provide information for comparison of Hg species accumulation by mosquitofish from water sources. However, to correct for the different exposure concentrations used in these studies, the accumulation model (Eq. 2) was modified. The following model is valid under the assumption that the elimination rate constant (k_e , per day) and uptake rate constant (k_{up} , per day) were independent of the exposure concentration.

$$C_t = (k_{up}/k_e)(C_w)(1 - e^{-(k_e + k_{up})t}) \quad (4)$$

where k_{up} = uptake rate constant (1/day),
 C_w = concentration in water ($\mu\text{g}/\text{ml}$).

All other parameters and variables in Eq. 4 were the same as those given in Eq. 2. With the units given in Eq. 2 and 4, k_{up} is equal to k_u/C_w . For convenience of scale, time units will be hours instead of days where indicated. Uptake rate constants for methylmercury in mosquitofish exposed to 0.1 $\mu\text{g Hg/L}$ were smaller (26 per hr) than those derived in the present study for mosquitofish exposed to 0.24 $\mu\text{g/L}$ of Hg (II) (167 per hr). The rate constants derived from the data of Schoper for mercuric chloride and Hg^0 were 115 and 136 per hr, respectively. The two inorganic Hg species are taken up more rapidly than methylmercury.

Uptake of Hg (II) was correlated significantly with mosquitofish size; however, there was considerable variation between individuals. The variation precluded development of a statistical model for size-dependent Hg accumulation. In contrast, Zn uptake in mosquitofish displayed a clear power relation to size.

Elimination

Methylmercury accumulation (Huckabee *et al.* 1975, Figure 3) from water containing 0.1 $\mu\text{g Hg/L}$ was modeled and the estimate of k_e was 0.11 ± 0.04 per day. This was slower than that derived in the present study (0.53 ± 0.14 per day). Data from Schoper (1974) suggested that Hg^0 was eliminated at approximately the same rate as the Hg accumulated as Hg (II) (0.14 ± 0.01 and 0.13 ± 0.01 per day, respectively). Generally, the two inorganic Hg species were eliminated faster than methylmercury.

Elimination kinetics for Hg taken up as Hg (II) were determined statistically to be independent of mosquitofish size. If any size-dependence for elimination were present, it was insignificant relative to other differences between individuals. Wide individual variation among mosquitofish in methylmercury accumulation kinetics was also noted by Huckabee *et al.* (1975). Newman and Mitz (1988) reported significant effects of mosquitofish size on the slow elimination of Zn by mosquitofish. However, these authors also found considerable variation in elimination between individuals that could not be attributed to fish size. This relation for Zn elimination conformed to a power model as reported for size-dependent methylmercury elimination in goldfish (*Carassius auratus*) by Sharpe *et al.* (1976).

Accumulation

Equilibrium concentrations of Hg in mosquitofish (k_u/k_e) averaged 0.64 $\mu\text{g/g}$ dry wt when the standard model (Eq. 2) and Richards' model (Eq. 3) were

used to fit these data. Use of Eq. 2 alone to estimate the average equilibrium concentration for the 19 typical mosquitofish produced a similar value, 0.63 $\mu\text{g/g}$ dry wt. Estimates using Eq. 2 for the two atypical fish had large standard errors (0.99 ± 0.21 and 1.88 ± 1.48 $\mu\text{g/g}$ dry wt). The estimates for these two fish were improved (0.79 ± 0.04 and 0.99 ± 0.03 $\mu\text{g/g}$ dry wt) when the Richards' model was used. The smaller residuals from the Richards' model and the standard errors for the equilibrium concentration estimates suggested that Eq. 3 was more effective in describing Hg accumulation in the two atypical mosquitofish than Eq. 2.

Smaller fish had generally higher equilibrium Hg concentrations than larger fish. The size-dependence of equilibrium concentration was produced primarily by size-dependent uptake. Although there was a significant, size-dependence of Zn elimination in mosquitofish (Newman and Mitz 1988), uptake also dominated the size-dependence of Zn body burden in mosquitofish; thus, size-dependent uptake rate was the dominant factor influencing size-dependent body burden for two, group IIB metals.

Acknowledgments. This work was supported by contract DE-AC09-76SR00-819 between the U.S. Department of Energy and the University of Georgia's Savannah River Ecology Laboratory. The authors gratefully acknowledge the advice and suggestions of Dr. John E. Pinder, III, throughout the tenure of this study. Dr. Margaret E. Mulvey and Mr. Douglas Martinson provided critical reviews of the manuscript. Jan Hinton typed the manuscript. The Figures were produced using Version 3.00 of Sigma Plot.

References

- APHA (1975) American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for The Examination of Water and Wastewater. 14th ed, New York: APHA
- Baker CR, Dunaway PB (1969) Retention of ^{137}Cs as an index of metabolism in the cotton rat (*Sigmodon hispidus*). Health Phys 16:227-230
- Beauchamp JJ, Olson JS (1973) Corrections for bias in regression estimates after logarithmic transformation. Ecology 54:1403-1407
- Bence JR, Murdoch WW (1986) Prey size selection by the mosquitofish: Relation to optimal diet theory. Ecology 67(2):324-336
- Boudou A, Delarche A, Ribeyre F, Marty R (1979) Bioaccumulation and bioamplification of mercury compounds in a second level consumer, *Gambusia affinis*—Temperature effects. Bull Environ Contam Toxicol 22:813-818
- Boyd CE, Ferguson DE (1964a) Spectrum of cross-resistance to pesticides in the mosquito fish, *Gambusia affinis*. Mosquito News 24(1):19-21
- , —— (1964b) Susceptibility and resistance of mosquitofish to several insecticides. J Econ Entomol 57(4):430-431

- Boyden CR (1974) Trace element content and body size in molluscs. *Nature* (London) 251:311-314
- (1977) Effect of size on metal content of shellfish. *J Mar Biol Assoc UK* 57:675-714
- Brisbin IL, Jr, White GC, Bush PB (1986) PCB intake and the growth of waterfowl: Multivariate analyses based on a reparameterized Richards sigmoid model. *Growth* 50:1-11
- Brown KL (1985) Demographic and genetic characteristics of dispersal in the mosquitofish, *Gambusia affinis* (Pisces: Poeciliidae). *Copeia* 1985(3):597-612
- Burrows WD, Krenkel PA (1973) Studies on uptake and loss of methylmercury-203 by bluegills (*Lepomis macrochirus* Raf.). *Environ Sci Technol* 7(13):1127-1130
- Cech JJ, Jr, Massingill MJ, Wragg TE (1980) The food demands of mosquitofish, *Gambusia affinis*: Proceedings and Papers of The Forty-eighth Annual Conference of The California Mosquito and Vector Control Association, Jan. 20-23, Los Angeles, CA
- Cech JJ, Jr, Massingill MJ, Vondracek B, Linden AL (1984) Respiratory metabolism of mosquitofish, *Gambusia affinis*: Effects of temperature, dissolved oxygen and sex difference. *Environ Biol Fishes* 13(4):297-307
- Cossa D, Bourget E, Pouliot D, Piuze J, Chanut JP (1980) Geographical and seasonal variation in the relationship between trace element content and body weight in *Mytilus edulis*. *Mar Biol* 58:7-14
- EPA: US Environmental Protection Agency (1978) Environmental Monitoring Series. Quality Assurance Guidelines for Biological Testing. Environmental Monitoring and Support Laboratory, Cincinnati, OH, EPA-600/4-78-043
- Fagerstrom T (1977) Body weight, metabolic rate and trace substance turnover in animals. *Oecologia* (Berlin) 29:99-104
- Hannerz L (1968) Experimental investigations on the accumulation of mercury in water organisms. Inst. of Freshwater Research Report No. 48, Fishery Board of Sweden, Lund, pp 120-176
- Hill G (1984) Microcomputer Assisted Quality Assurance, Bakersfield, CA.: Hill Inc
- Hughes TH, Matis JH (1984) An irreversible two-compartment model with age-dependent turnover rates. *Biometrics* 40:501-505
- Huckabee JW, Goldstein RA, Janzen SA, Woock SE (1975) Methylmercury in a freshwater foodchain. Symposium Proceedings: International Conference on Heavy Metals in The Environment. Vol. II, Part 1, Toronto, Ontario, Canada
- Kennedy PK, Smith ML, Zimmerman EG, Chesser RK, Smith MH (1986) Biochemical genetics of mosquitofish. V. Perturbation effects on genetic organization of populations. *Copeia* 1986(4):937-945
- Krumholz LA (1948) Reproduction in the western mosquitofish, *Gambusia affinis affinis* (Baird and Girard), and its use in mosquito control. *Ecol Monogr* 18(1):343
- Matis JH, Wehrly TE, Gerald KB (1983) The statistical analysis of pharmacokinetic data. In: *Lecture Notes in Biomathematics: Tracer Kinetics and Physiologic Modeling*. Editor: S. Levin, Springer-Verlag, NY
- Matis JH (1972) Gamma time-dependency in Blaxter's compartmental model. *Biometrics* June 1972:597-602
- McKone CE, Young RG, Bache CA, Lisk DJ (1971) Rapid uptake of mercuric ion by goldfish. *Environ Sci Technol* 5:1138-1139
- Newman MC, McIntosh AW (1983) Lead elimination and size effects on accumulation by two freshwater gastropods. *Arch Environ Contam Toxicol* 12:25-59
- Newman MC, Mitz SV (1988) Size dependence of zinc elimination and uptake from water by mosquitofish, *Gambusia affinis* (Baird and Girard). *Aquatic Toxicol* 12:17-32
- Norstrom RJ, McKinnon AE, DeFreitas ASW (1976) A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). *J Fish Res Board Can* 33:248-267
- Pentreath RJ (1976a) The accumulation of inorganic mercury from seawater by the plaice, *Pleuronectes platessa* L. *J exp mar Biol Ecol* 24:103-119
- Pentreath RJ (1976b) The accumulation of mercury from food by the plaice, *Pleuronectes platessa* L. *J exp mar Biol Ecol* 25:51-65
- Peters EL (1986) Radiocesium kinetics in the yellow-bellied turtle, *Pseudemys scripta*. M. Thesis, University of Georgia, Athens, GA
- Pulliam HR, Barrett GW, Odum EP (1969) Bioelimination of tracer ^{65}Zn in relation to metabolic rates in mice. *Proc 2nd Nat. Symp. Radioecology, CONF-670503*, pp. 725-730, Ann Arbor, MI
- Richards F (1959) A flexible growth function for empirical use. *J Exper Bot* 10:290-300
- Saiki MK (1987) Relation of length and sex to selenium concentration in mosquitofish. *Environ Pollut* 47:171-186
- SAS Institute (1985) SAS User's Guide: Statistics. SAS Institute, Inc, Cary, NC
- Schooper NJ (1974) The uptake, biotransformation and elimination of elemental mercury by fish. M. Thesis, University of Georgia, Athens, GA
- Sharpe MA, deFreitas ASW, McKinnon AE (1976) The effect of body size on methylmercury clearance by goldfish (*Carassius auratus*). *Environ Biol Fish* 2(2):177-183
- Shin EB, Krenkel PA (1976) Mercury uptake by fish and bi-methylation mechanisms. *J WPCF* 48(3):473-501
- Spacie A, Hamelink JL (1985) Chapter 17. Bioaccumulation. In: *Fundamentals of Aquatic Toxicology*, Rand, GM, Petrocelli, SR (eds) Hemisphere Pub Corp, New York, p 666
- Strong CR, Luoma SN (1981) Variations in the correlation of body size with concentrations of Cu and Ag in the bivalve, *Macoma balthica*. *Can J Fish Aquat Sci* 38:1059-1064
- Wallen IE, Greer WC, Lasater R (1957) Toxicity to *Gambusia affinis* of certain pure chemical in turbid waters. *Sewage Ind Wastes* 29:695-711
- Watling RJ, McClurg TP, Stanton RC (1981) Relation between mercury concentration and size in the mako shark. *Bull Environ Contam Toxicol* 26:352-358
- Williams DR, Giesy JP, Jr (1978) Relative importance of food and water sources to cadmium uptake by *Gambusia affinis* (Poeciliidae). *Environ Res* 16:326-332
- Williamson P (1980) Variables affecting body burdens of lead, zinc and cadmium in a roadside population of the snail, *Cepaea hortensis* (Muller). *Oecologia* (Berlin) 44:213
- Wurtsbaugh WA, Cech JJ, Jr (1983) Growth and activity of juvenile mosquitofish: Temperature and ration effects. *Trans Amer Fisheries Soc* 112:653-660