Aquatic Toxicology, 12 (1988) 17–32
Elsevier

AQT 00255

Size dependence of zinc elimination and uptake from water by mosquitofish *Gambusia affinis* (Baird and Girard)

Michael C. Newman and Stephen V. Mitz

Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29801, U.S.A.

(Received 30 September 1986; revision received 26 May 1987; accepted 12 June 1987)

Zinc-elimination rate constants decreased with increasing fish size as described in the equation k_e = 0.001 (dry wt.)$^{-0.42}$. One compartment displaying a median biological half-life of 215 days was identified. Additional compartments were not discernable using backstripping methods. Zinc-uptake rates from water also decreased with increasing fish size. The equation describing this relationship was k_u = 0.029 (dry wt.)$^{-0.90}$. Size-dependent relationships for body burdens of Zn in *G. affinis* were derived by combining the effects of size-dependent uptake from water and elimination kinetics. A non-equilibrium approach incorporating fish size and duration of exposure was used to generate relationships between fish size and Zn-body burden with exponents (*b* values) less than 1. As duration of exposure increased, the *b* values for the size-dependent body-burden relationships also increased.

Key words: *Gambusia affinis*; Zinc; Fish; Uptake; Elimination; Size.

INTRODUCTION

The degree of contamination in aquatic environments is frequently assessed by comparing contaminant concentrations in associated biota. However, bioaccumulation is influenced by factors other than the degree of contamination, such as temperature and salinity (Unlu and Fowler, 1979), organic and inorganic ligands (Gjessing, 1981; Bingham et al., 1984), season (Cossa et al., 1980), feeding behavior and microhabitat utilization (Newman and McIntosh, 1982), and diet (Graney et al., 1984). These complicating factors often remain poorly defined in bioaccumulation studies. One such factor, which varies within and between populations, is organism size. Despite the importance of size in influencing contaminant body burden (Eber-

Correspondence to: M.C. Newman, Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29801, U.S.A.

hardt, 1967; Reichle and Van Hook, 1970; Cossa et al., 1980; Williamson, 1980; Watling et al., 1981; Kumagai and Saeki, 1983), few studies have attempted to quantify this relationship or elucidate the underlying mechanisms.

Boyden (1974, 1977) used power functions to fit contaminant body burdens of field-sampled invertebrates to animal size. Most size-dependent relationships for contaminant body burdens studied by Boyden (1977) fit into one of two classes displaying exponents (*b* values) of either 1 or less than 1. Boyden suggested that the body burden was determined by the amount of tissue available to bind a trace element for the relationship with $b = 1$. A relationship with a *b* value less than 1 (averaging 0.77) implied a connection with metabolism, 'as many metabolic processes display a similar relation to body weight' (Boyden, 1974). However, Fagerstrom (1977) argued that a *b* value of 1, not 0.75, was indicative of direct linkage of metabolic rate (a flux) to size-dependent contaminant body burden (a state) under steady-state conditions. In a second study, Boyden (1977) suggested that a linkage between metabolic rate and rates of uptake or loss could produce this type of coefficient. Other workers have suggested similar linkages between metabolic rate and components of the accumulation process for a variety of elements and species (Eberhardt, 1967; Holleman et al., 1971; Anderson and Spear, 1980; Williamson, 1980; Whicker and Schultz, 1982a,b; and Peters, 1986). Alternate proposed mechanisms controlling relationships with *b* values less than 1 include the following: (1) more rapid short-term uptake by smaller individuals (Strong and Luoma, 1981); (2) changes in surface:volume ratio during growth (Newman and McIntosh, 1983; Smock, 1983); (3) size-dependent feeding behavior (Fagerstrom, 1977); (4) size-dependent concentrations of some biochemical entity involved in accumulation kinetics, such as size-dependent enzyme concentrations (Hoppe-Seyler, 1964); or (5) the interactions of many factors (Williamson, 1980).

The purpose of the present study was to develop an animal model for the size dependence of trace-element accumulation. Size-dependent Zn-accumulation kinetics from water by the mosquitofish *Gambusia affinis* was selected as the model. The equations describing the size dependence of the uptake-rate and elimination-rate constant were then used to generate size-dependent body-burden relationships.

MATERIALS AND METHODS

Gambusia

G. affinis were collected at the Pen Branch Creek delta on the Savannah River Plant (Aiken, South Carolina, U.S.A.) during April and May 1985. The fish were treated with 2 mg/l $KMnO_4$ for 4 days to prevent disease outbreaks. Fish were kept in 38-l aquaria in an Environator controlled temperature growth chamber to maintain a water temperature of approximately 18°C during acclimation. Water for all of the studies was reconstituted very soft freshwater (RVSFW) (USEPA, 1978),

modified by adding 2 mg NaCl/l and 6 mg $CaCl_2 \cdot H_2O$/l to make the concentrations of Na, Cl, and Ca comparable to concentrations measured at the Pen Branch Creek swamp delta site.

Gamma-counting protocol

Prior to beginning the elimination phase of the experiment, a ^{65}Zn-labeled fish was passed through consecutive rinses in five 100-ml aliquots of RVSFW. Ten ml of each rinse-water were then counted for ^{65}Zn activity. No rinse-water activity was greater than background levels after the first rinse; therefore, three consecutive rinses in 250 ml of RVSFW each rinse was determined to be sufficient to avoid any transfer of activity associated with exposure tank waters.

The effect on geometry changes resulting from fish movement during gamma-activity measurements was examined. One medium-sized fish that had been exposed for 75 days to the conditions described below for the 38-l exposure tank (elimination experiment) was counted five times alive (variable geometry) and immediately after death (fixed geometry) in 10 ml of fresh RVSFW. There was no significant difference in gross counts (12 min) derived from the variable and fixed geometries. Therefore, the effect of fish movement on counting efficiency was sufficiently small to allow precise counting of live fish.

Stable zinc in fish

Frozen fish were lyophilized for 18 h and weighed in acid-washed polyethylene vials. Each fish was placed into a 10-ml TFE Teflon beaker containing 4–6 ml Ultrex-concentrated nitric acid, covered with a TFE Teflon lid and left for 1 h at room temperature. Next, the covered beakers were refluxed at 80°C for 4 h. Samples were evaporated to approximately 2 ml, cooled, and volumetrically diluted to either 5, 10, or 50 ml, depending on total weight of fish in each beaker. Two digestion blanks and eight US EPA 'Quality Control Samples, Metals in Fish' samples were digested. The mean and standard deviation for the eight standard-material samples (42.4 $\pm$ 6.6 μg Zn/g dry wt.) were comparable to the values reported by the US EPA (43.6 $\pm$ 4.1 μg Zn/g dry wt.). Each sample was analyzed by the method of standard additions.

Elimination kinetics

Although the elimination rate (k_e) and uptake rate constants (k_u) have been derived from accumulation curves in numerous studies, this approach could not be used as the bias associated with fish growth during the necessarily long exposure period, as indicated by the work of Willis and Jones (1977), would invalidate the results. Instead, elimination rate constants and uptake rates for various-sized mosquitofish

were determined in separate experiments. Minimal bias was expected from this approach as both approaches produce similar results (Cutshall, 1974).

Approximately 30 assorted-sized *G. affinis* were held in four tanks during 77 days of exposure to 50 μg Zn/l and 0.1 μCi ^{65}Zn/l nominal concentration and activity, respectively. An additional 15 assorted-sized fish were kept in separate aquaria and not exposed to Zn or ^{65}Zn. Two of the tanks had capacities of 3.8-l while two had capacities of 38 l. Exposures were conducted in a Precision illuminated low-temperature incubator at approximately 18°C. Water temperature in the 3.8-l tank containing the smaller experimental fish was 18.5 ± 1.7°C (n = 65) while the water temperature in the 38-l tank containing the larger experimental fish was 18.4 ± 1.7°C. Water temperatures in the tanks holding the small and large control fish were 18.6 ± 2.1 and 18.3 ± 1.7°C, respectively.

The mean ^{65}Zn activities in the tanks were measured prior to daily water replacement and 2–3 h after water replacement. Identical mean activities before and after water replacement (0.09 ± 0.02 μCi/l; n = 63) were measured in the small tank holding ^{65}Zn-exposed fish. ^{65}Zinc activities in the water of the large exposure tank were 0.07 ± 0.02 μCi/l (n = 63) before water replacement and 0.08 ± 0.02 μCi/l (n = 63) after water replacement. There was no apparent loss of dissolved ^{65}Zn from the water between renewals. The water in the small and large tanks holding control (nonexposed) fish never had ^{65}Zn activities above the counting background (n = 63).

On day 0 of the elimination phase, 20 of the Zn/^{65}Zn-exposed fish and 5 nonexposed fish were counted for ^{65}Zn activity with a Beckman Gamma 8000 gamma counter. Individual fish were captured in a nylon net and rinsed sequentially as previously described. Water in the three beakers was replaced with fresh RVSFW after being used to rinse five fish. Each fish was transferred to a plastic scintillation vial containing 10–20 ml of RVSFW and counted for 12 min. Only 1.115-MeV gamma photons were counted.

Immediately after completion of gamma activity measurements, each fish was returned to an individual Plexiglas chamber (0.2–1.7 l depending on fish size), contained within a larger Plexiglas (24 l) tank. Each individual chamber had a 0.75-mm^2 mesh Nytex bottom raised 3 mm off the tank bottom to allow free exchange of water between chambers. Fish were assayed for ^{65}Zn activity after 0, 0.5, 1, 2, 4, 6, 9, 13, 17, 21, 25, and 31 days of depuration. Water samples for ^{65}Zn-activity determinations were collected daily prior to an approximately 70% partial water change. Fish were fed Tetra Guppy Special Diet (mean ± standard deviation = 111 ± 3 μg Zn/g dry wt.; n = 3) each day after they had been counted for ^{65}Zn activity.

Uptake kinetics

The exposure system consisted of individual Plexiglas chambers contained within a larger Plexiglas tank as described in the elimination kinetics methods. Twenty fish

were exposed for 30 days to 50 μg Zn/l and 0.1 μCi ^{65}Zn/l nominal concentration and activity, respectively. Five assorted-sized *G. affinis* were exposed to 50 μg Zn/l in a separate Plexiglas exposure system. The fish were counted for ^{65}Zn-gamma activity after 0, 1, 2, 4, 11, 15, 19, 23, 27, and 30 days of exposure. Counting procedures were the same as previously described for the elimination-kinetics study.

Water chemistry

Dissolved oxygen concentrations were determined by the azide modification Winkler titration method (APHA, 1975). Total alkalinity was measured by potentiometric titration (APHA, 1975). Specific conductance was measured at 25°C using a cell with a constant of 0.1 cm^{-1}. Chloride and SO_4 concentrations were measured using a Dionex 2020i-ion chromatograph with a conductivity detector and HPIC-AS4 separator column (2.8 mM $NaHCO_3$/2.3 mM Na_2CO_3 eluant). K, Na, Ca, and Mg concentrations were measured by flame atomic-absorption spectrophotometry (Hitachi 180–80 atomic-absorption spectrophotometer with Zeeman background correction). Zinc concentrations were measured by graphite furnace atomic-absorption spectrophotometry. Water samples and 60-ml aliquots of deionized water (filter blanks) were drawn through 0.45-μm HAWP04700 Millipore filters, acidified with 0.5 ml of Ultrex-concentrated nitric acid per 60 ml of sample and analyzed by flameless atomic-absorption spectrophotometry using a Hitachi 180–80 spectrophotometer.

Water samples from the exposure tank were collected before and after a daily 70% partial change of water. ^{65}Zn activity in aqueous samples was measured using a Beckman Gamma 8000 gamma counter. Instrument efficiency was calculated each counting day.

Elimination model

The decrease in ^{65}Zn activity in each fish due to radioactive decay and zinc elimination was modeled with the following equation:

$$A_t = A_0 e^{-(k_e + \lambda)t}, \quad (1)$$

where A_t = ^{65}Zn activity at time t (pCi/g dry wt.), A_0 = ^{65}Zn activity at time 0 (pCi/g dry wt.), t = duration of depuration (days), k_e = elimination rate constant (day^{-1}), λ = radioactive decay constant (day^{-1}).

The elimination rate constant (k_e) for each fish was estimated by subtraction of the radioactive decay rate from the slope of the least-squares regression equation of ln pCi ^{65}Zn/g dry wt. vs. the number of days of depuration. All calculations were performed using the SAS-software package (SAS Institute, 1982). The relationship

between k_e and fish size was estimated using linear regression analysis of log k_e vs. log fish dry wt. Bias corrections were made using the methods of Beauchamp and Olson (1973).

Uptake model

The changes in stable Zn concentrations were estimated using the ratio of ^{65}Zn activity to stable Zn concentration in the water. Uptake rates were derived assuming the following accumulation model:

$$C_t = C_e(1-e^{-(k_e + \lambda)t}), \tag{2}$$

or

$$C_t = (k_u/k_e)\ (1-e^{-(k_e + \lambda)t}), \tag{3}$$

where C_t = Zn concentration at time t (μg/g dry wt.), C_e = Zn concentration at equilibrium (μg/g dry wt.), t = duration of exposure (day), k_e elimination rate constant (day^{-1}), λ = radioactive decay constant (day^{-1}), k_u = uptake rate (μg/g dry wt./day).

The uptake rate (k_u) for each fish was estimated using Eqn. 3 and a nonlinear regression program in the SAS statistical package (SAS Institute, 1982). Two approaches were taken to estimate the k_e values used in the accumulation model because the exponent for the relation between k_e and fish size had a relatively large SE. The first approach used the median k_e value for all fish in the elimination kinetics experiment. The use of the median k_e implies the assumption that elimination rate is independent of size. In the second approach, the estimate of the k_e value for an individual fish was calculated using a linear regression model for the elimination experiment results (k_e vs. fish wt.). The relationship between log k_u and log fish wt. was estimated using the linear regression procedure in the SAS statistical package. Bias corrections were made using the methods of Beauchamp and Olson (1973).

RESULTS

Elimination model

The water quality during the elimination phase of this experiment is summarized in Table I. Minor differences (4.5%) between the sum of cations (0.560 meq/l) and sum of anions (0.469 meq/l) indicated acceptable ionic balance.

Fish size ranged from 0.016 to 0.230 g dry wt. at the end of the experiment. The greatest stable Zn concentration (507 μg/g dry wt.) was noted in a 0.016 g fish while

TABLE I

Water-quality characteristics for ^{65}Zn elimination and uptake experiments.

Variable	Elimination experiment			Uptake experiment		
	Mean	SD	N[a]	Mean	SD	N[a]
Temperature (°C)						
Exposure tank	16.8	1.6	25	18.7	1.7	23
Control tank				18.5	1.6	24
Specific conductance (μmhos/cm)	62.7	0.3	15	65.9	2.4	15
Dissolved oxygen (mg O_2/l)	7.3	0.5	31	8.1	0.3	28
Percentage oxygen saturation	74	5	31	85	2	28
pH[b]	6.88	6.67–7.01	32	6.81	6.61–7.15	30
Calcium (mg/l)	3.7	0.7	32	4.5	0.7	30
Magnesium (mg/l)	1.44	0.02	32	1.47	0.05	30
Sodium (mg/l)	5.1	0.3	32	4.2	0.3	30
Potassium (mg/l)	1.46	0.05	32	1.55	0.03	30
Total alkalinity (mg/l as $CaCO_3$)	7.4	0.4	32	5.5	0.3	30
Chloride (mg/l)	6.2	1.1	32	7.1	0.3	30
Sulfate (mg/l as SO_4)	10.6	0.6	32	11.9	0.4	30
Dissolved Zinc (μg/l)	1.9	0.6	27	59.8	19.5	30
$^{65}Zinc$ (pCi/l)	Bkg[c]		32	0.106[d]	0.008	23

[a] Number of samples analyzed. [b] Median and range. [c] Indistinguishable from background counts. [d] Measured in the exposure tank. Activities in the control tank were indistinguishable from background counts.

the largest fish (0.2309) had the lowest stable Zn concentration (37 μg/g dry wt.). The mean Zn concentration of the experimental animals was 202 ± 70 μg/g dry wt. (n = 18).

No detectable ^{65}Zn activity (detection limit: 24.5 pCi) was measured in any control fish during the period of depuration. ^{65}Zn activity in exposed fish decreased very slowly during the depuration period. Fig. 1 presents the change in activity in four representative experimental fish during depuration. Elimination rate constants (k_e) were calculated by fitting these data for all fish to Eqn. 1. There was a general decrease in k_e with increasing fish size (Fig. 2) except the largest fish (inverted triangle in Fig. 1). The largest fish was a gravid female judged to have anomalous elimination kinetics. The unusually high elimination rate constant measured for this fish could have resulted from the Zn kinetics of the developing embryos as well as changes in the female relative to her gravid condition. The abrupt slope changes at the beginning and end of the depuration period suggest that parturition during these periods could also have contributed to the anomaly. Bias-corrected linear regression analysis of the elimination rate constants and fish size yielded the relationship k_e = 0.001 (dry wt.)$^{-0.42}$ (Table II). The SE of the exponent estimate was large (0.14, n = 17). The biological half-life of Zn (t_{50}) was calculated from these elimination rate constants using the equation:

$$t_{50} = 0.693/k_e. \quad (4)$$

The mean and SD of t_{50} of these fish were 215 ± 111 days when the t_{50} for the one anomalous fish was omitted from the data set. Generally, the smaller fish had shorter t_{50} values for Zn. However, the large gravid female had a t_{50} of 90 days.

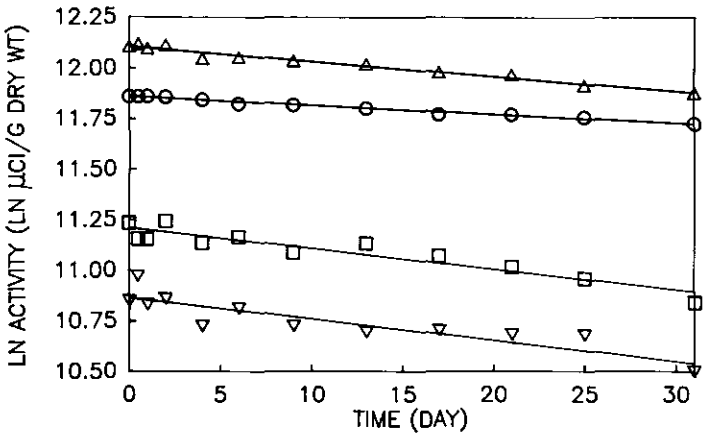

Fig. 1. Representative depuration curves for four experimental fish. The fish selected weighed 0.019 (square), 0.033 (triangle), 0.051 (circle), and 0.230 (inverted triangle) g dry wt. at the termination of the experiment. The least-squares estimates for the model used to derive the elimination rate constant from the date are shown as solid lines.

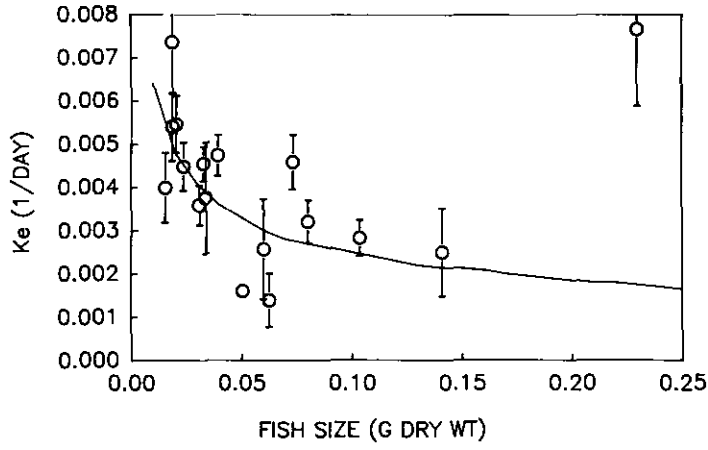

Fig. 2. The relationship between elimination rate constant and fish size. SE for each estimate is indicated by vertical error bars. The relationship estimated by regression analysis is shown as a solid line.

Uptake model

For reasons of radiation safety, only water temperature and ^{65}Zn activity were measured in the exposure tank during the study (Table I). Water quality of the control tank water was assumed to reflect that in the exposure tank as both tanks were treated similarly. The mean ^{65}Zn activity during the exposure period was 0.106 μCi/l in the exposure tank and background activity in the control tank. The mean dissolved Zn concentration was 58.9 μg/l in the control tank. The sum of the cations (0.568 meq/l) differed from the sum of anions (0.503 meq/l) by 3% indicating acceptable ionic balance.

Fish size ranged from 0.006 to 0.213 g dry wt. at the end of the exposure period. Mean and SD of stable Zn concentration in the experimental fish were 215 $\pm$ 49 μg/g dry wt. ($n=20$). Stable Zn concentrations in the control fish were 207 $\pm$ 26 μg/g dry wt. ($n=5$). No detectable ^{63}Zn activity was measured in the control fish during the exposure period.

Typical accumulation data for three fish are presented in Fig. 3. Uptake data were fit to Eqn. 3 using the nonlinear regression procedures of the SAS statistical software package (Fig. 4). An estimate of k_e was provided for each fish using the relationship between fish size and k_e generated in the elimination experiment. Although the exponent in the elimination model was significantly different from 0 (Table II), the confidence interval for the exponent of this relationship was wide. Therefore, the predictive value of this relationship is poor. For this reason, k_u values were also derived using the median value from the elimination experiment instead of the size-dependent estimates of k_e. The k_u values derived assuming size dependence of k_e ranged from 0.12 to 2.42 μg Zn/g dry wt/day. The k_u values from the model with

TABLE II

Regression equations for size-dependent elimination and uptake (k = a dry wt.^{b})

Model	Estimate of a (SE)	Estimate of b (SE)	n^a	F value	$P>F^b$	r^2
Elimination-rate constant vs. dry wt. (log k_e vs. log dry wt.)	0.001 (<0.001)	−0.42 (0.14)	17	8.88	0.010	0.39
Uptake rate vs. dry wt. (log k_u vs. log dry wt.)						
(a) Assuming k_e is size-dependent	0.029 (0.002)	−0.90 (0.07)	20	160.82	0.0001	0.90
(b) Assuming k_e is size-independent	0.057 (0.005)	−0.69 (0.07)	20	96.42	0.0001	0.84

[a] n = number of samples.

[b] probability of obtaining the F value under the 0 hypothesis that the slope = 0.

the median k_e value placed into the equation ranged from 0.18 to 1.80 μg Zn/g dry wt/day. The smaller fish had larger uptake rates than the larger fish under both sets of assumptions. Uptake rates were expressed on a μg/fish/day basis prior to linear regression analysis (log uptake rate vs. log g dry wt.) to avoid the presence of fish wt. in the independent and dependent variables. The results were corrected for bias

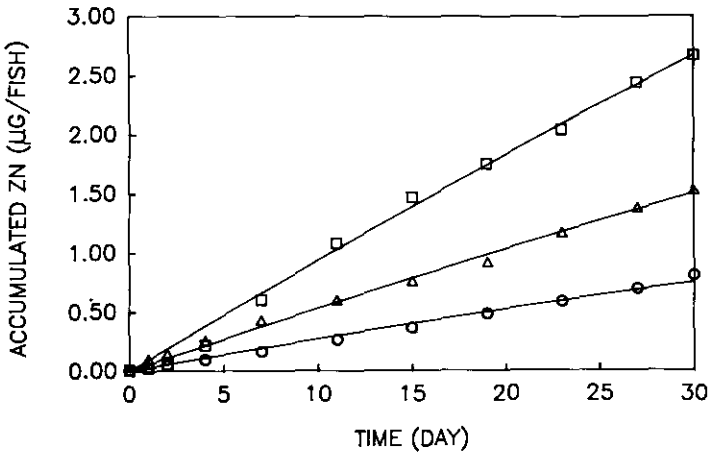

Fig. 3. Representative accumulation curves for three experimental fish. The fish selected weighed 0.009 (circle), 0.065 (triangle) and 0.213 (square) g dry wt. at the termination of the experiment. The least-squares estimates for the model used to derive uptake rates from these data are shown as solid lines.

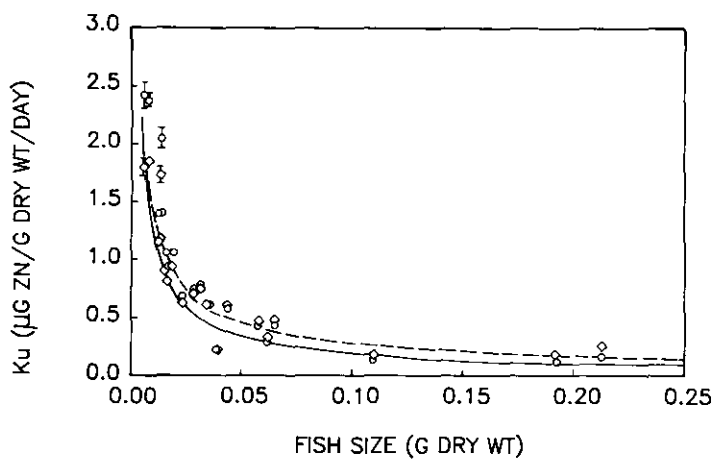

Fig. 4. The relationship between uptake rate and fish size. SE for each point is indicated by vertical lines; however, most error bars were sufficiently small as to be contained within the symbols denoting data points. The relationships estimated for uptake rate and fish size assuming size-dependent (solid line) or size-independent (dashed line) elimination rate constants are also shown. Estimates of uptake rate generated under the assumptions of size-independent or size-dependent elimination rate constants are denoted by circles and diamonds, respectively.

(Beauchamp and Olson, 1973) and then converted to a μg/g dry wt/day bais. When the size-dependent k_e values were used in Eqn. 3, the following regression model was obtained, $k_u = 0.029$ (dry wt.)$^{-0.90}$ (Table II). The regression model generated when the median k_e was used was $k_u = 0.057$ (dry wt.)$^{-0.69}$.

DISCUSSION

Elimination

Willis and Jones (1977) found that 68 days of exposure to ^{65}Zn in food and water was adequate for uniform labeling of *G. affinis* with ^{65}Zn. These results suggest that the 77 days of ^{65}Zn exposure used in the present study was adequate for labeling the major compartments. However, as exposure was only from water, uniform labeling was not assumed in the present study.

Willis and Jones (1977) noted three mathematical compartments for Zn elimination in adult *G. affinis* (0.21 g wet wt.) exposed to ^{65}Zn in food and water. The elimination rate constants measured in the present study were similar to that of the slow compartment (Compartment 3) measured by Willis and Jones (1977). These investigators estimated that this compartment contained approximately 91% of the total Zn. Only one compartment was apparent for Zn elimination measured in the present study. Attempts to identify additional compartments using back-projection methodologies (Wagner, 1975) failed. In contrast to the present study, Willis and Jones (1977) exposed fish to ^{65}Zn in both food and water; therefore, one or both of these minor compartments with rapid turnover (Compartments 1 and 2) likely were associated with gut clearance or other mechanisms linked to accumulation from food. As the dominant compartment in both studies contained 91% (Willis and Jones) or 100% (present study) of the total body burden of ^{65}Zn and modes of exposure were not identical between the studies, a multiple compartment approach was deemed unwarranted and the minor rapid-turnover compartments identified by Willis and Jones (1973) were not incorporated into the model.

The mean t_{50} for Zn (215 days) was comparable to that derived for the major Zn compartment (235 days) identified by Willis and Jones (1977). As the time to equilibrium is determined by the k_e and smaller fish had larger k_e values than larger fish, the smaller fish approach equilibrium Zn concentrations faster than the larger fish. Using the following equation (Spacie and Hamelink, 1985),

$$t_{95} = -(\ln 0.05/k_e), \tag{5}$$

the estimated times to reach 95% of the equilibrium concentration for a small (0.01 g dry wt.) and large fish (0.25 g dry wt.) would be 468 and 1816 days, respectively. Given the long periods necessary to approach equilibrium concentrations and relatively short life span for these fish (1–2 y), it is invalid to develop models or ex-

planations for size-dependent Zn-body burdens for the mosquitofish based on assumptions of equilibrium conditions.

The size dependence of Zn elimination measured in this study was supported by the size-dependent elimination noted for other elements in a variety of animals (Eberhardt, 1967; Gallegos and Whicker, 1971; Fendley et al., 1977). The large SE associated with the estimate of the exponent for the relationship between k_e and fish size (g dry wt.) precludes comparison of the relationship to that for size-dependent metabolic rate.

Uptake

Small mosquitofish had clearly higher uptake rates from water than those of the larger mosquitofish. The exponent for the power equation relating uptake rate (μg Zn/g dry wt/day) to fish size was -0.90 (size-dependent elimination assumed) or -0.69 (size-independent elimination assumed). The b values for these relationships calculated as μg Zn/fish/day were 0.10 and 0.31, respectively. Interpretation of the relationship between k_u and fish size ($b = 0.10$ or 0.31) based solely on the ratio of gill surface available for uptake to fish wt. fails as the relationship between gill surface and fish wt. normally is described by a power function with an exponent of 0.5 to 1.0 (Hughes, 1984). Similarly, interpretation of these b values based on size-dependent metabolic rate alone fails as the relationship between metabolic rate and intraspecific animal size is normally described by a power function with an exponent greater than 0.60 (Prosser, 1973).

The estimates of uptake rates for small fish had wider SE than those of larger fish (Fig. 4). This tendency was attributed to the higher bias produced during faster growth of the smaller fish relative to the larger fish. Although present, size-dependent growth-rate effects did not strongly bias the results as evidenced by the small SE values associated with the estimated k_u values (Fig. 4) and the general shape of the accumulation curves during the period of accumulation (Fig. 3).

Accumulation

The relationships defining uptake rate from water and elimination rate constant in terms of mosquitofish size were used in Eqn. 3 to produce a model for size- and time-dependent accumulation of Zn from water. The model was solved for different size mosquitofish over a series of exposure durations. The purpose of this exercise was to demonstrate the type of relationships for size-dependent Zn concentrations which could be derived based only on this nonequilibrium model. Fish dry wt. ranging from 0.005 to 0.250 g and exposure times of 0–350 days were used in this exercise. The b values for size-dependent relationships increased slowly from 0.32 at 10 days of accumulation to 0.53 by 350 days of accumulation. Thus, the suggestion of Boyden (1974) that the b value for a particular trace element/species combination

remains constant was shown to be incorrect. Instead, the results supported the field data used by Cossa et al. (1980) and Strong and Luoma (1981) to refute the suggestion of constant *b* values.

The authors are aware that additional factors contribute to the size dependence of Zn-body burdens of *G. affinis*. Clearly, contributions of size-dependent uptake from food sources as well as other ecological, physiological and biochemical factors remain undefined in this study. Regardless, the combined effects of size-dependent uptake from water and elimination kinetics alone can produce relationships with *b* values less than 1. Additionally, this nonequilibrium model readily demonstrates one mechanism contributing to the variability in *b* values noted for field populations.

ACKNOWLEDGEMENTS

This research was supported by contract DE-AC09-76SR00-819 between the U.S. Department of Energy and the University of Georgia. The authors gratefully acknowledge the invaluable advice and suggestions of Dr. John E. Pinder, III, throughout the tenure of this study. Drs. Margaret E. Mulvey and F. Ward Whicker provided critical reviews of the manuscript. Jean Coleman drafted the figures and Marianne Reneau typed the manuscript. Sharon Presnell assisted in performing many water-quality analyses.

REFERENCES

Alberts, J.J., M.C. Newman and D.W. Evans, 1985. Seasonal variations of trace elements in dissolved and suspended loads for coal ash pond and pond effluents. Water Air Soil Pollut. 26, 111–128.

American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1975. Standard methods for the examination of water and wastewater. 14th edition, New York, 1193 pp.

Anderson, P.D. and P.A. Spear, 1980. Copper pharmacokinetics in fish gills – kinetics in pumpkinseed sunfish, *Lepomis gibbosus*, of different body sizes. Water Res. 14, 1101–1105.

Andrews, R.M. and F.H. Pough, 1985. Metabolism of squamate reptiles: allometric and ecological relationships. Physiol. Zool. 58, 214–231.

Apple, M.S. and M.A. Korostyshevskiy, 1980. Why many biological parameters are connected by power dependence. Theor. Biol. 85, 569–573.

Baker, C.E. and P.B. Dunaway, 1969. Retention of ^{134}Cs as an index to metabolism in the cotton rat (*Sigmodon hispidus*). Health Phys. 16, 227–230.

Beauchamp, J.J. and J.S. Olson, 1973. Corrections for bias in regression estimates after logarithmic transformation. Ecology 54, 1403–1407.

Bingham, F.T., G. Sposito and J.E. Strong, 1984. The effect of chloride on the availability of cadmium. Environ. Qual. 13, 71–74.

Boyden, C.R., 1974. Trace element content and body size in molluscs. Nature (London) 251, 311–314.

Boyden, C.R., 1977. Effect of size upon metal content of shellfish. J. Mar. Biol. Assoc. U.K. 57, 675–714.

Cossa, D., E, Bourget, D. Pouliot, J. Piuze and J.P. Chanut, 1980. Geographical and season variations in the relationship between trace element content and body weight in *Mytilus edulis*. Mar. Biol. 58, 7–14.

Eberhardt, L.L., 1967. Relationship of Cesium-137 half-life in humans to body weight. Health Phys. 13, 88–91.

Fagerstrom, T., 1977. Body weight, metabolic rate and trace substance turnover in animals. Oecologia (Berlin) 29, 99–104.

Fendley, T.T., M.N. Manlove and I.L. Brisbin, Jr., 1977. The accumulation and elimination and radiocesium by naturally contaminated wood ducks. Health Phys. 32, 415–422.

Gallegos, A.F. and F.W. Whicker, 1971. Radiocesium retention by rainbow trout as affected by temperature and weight. Proc. 3rd Nat. Symp. Radioecology, Oak Ridge, Tennessee, CONF-710501-P1, pp. 361–371.

Gjessing, E.T., 1981. The effect of aquatic humus on the biological availability of cadmium. Arch. Hydrobiol. 91, 144–149.

Graney, R.L., Jr., D.S. Cherry and J. Cairns, Jr., 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Water Res. 18, 833–842.

Holleman, D.F., J.R. Luick and F.W. Whicker., 1971. Transfer of radiocesium from lichen to reindeer. Health Phys. 21, 657–666.

Hoppe-Seyler, F., 1964. Handbuch der Physiologisch- und Pathologisch-chemischen Analyse. Vol. 6, Berlin, pp. 255.

Hughes, G.M., 1984. Measurement of gill area in fishes: practices and problems. J. Mar. Biol. Assoc. U.K. 64, 637–655.

Kumagai, H. and K. Saeki, 1983. The variations with growth in heavy metal contents of rock shell. Bull. Jpn. Soc. Sci. Fish. 49, 1917–1920.

Newman, M.C. and A.W. McIntosh, 1983. Lead elimination and size effects on accumulation by two freshwater gastropods. Arch. Environ. Contam. Toxicol. 12, 25–59.

Peters, E.L., 1986. Radiocesium kinetics in the yellow-bellied turtle, *Pseudemys scripta*. M. thesis, University of Georgia.

Prosser, C.L., 1973. Comparative animal physiology. W.B. Saunders Co., Philadelphia, pp. 966.

Pulliam, H.R., G.W. Barrett and E.P. Odum, 1969. Bioelimination of tracer ^{65}Zn in relation to metabolic rates in mice. Proc. 2nd Nat. Symp. Radioecology, CONF-670503, pp. 725–730.

Reichle, D.E., 1968. Relation of body size to food intake, oxygen consumption, and trace element metabolism in forest floor arthropods. Ecology 49, 538–542.

Reichle, D.E. and R.I. Van Hook, Jr., 1970. Radionuclide dynamics in insect food chains. Manit. Entomol. 4, 22–32.

SAS Institute, 1982. SAS user's guide: statistics. SAS Institute Inc., Cary, North Carolina, pp. 584.

Schmidt-Nielsen, K., 1975. Scaling in biology: the consequences of size. Exp. Zool. 194, 287–307.

Smock, L.A., 1983. Relationships between metal concentrations and organism size in aquatic insects. Freshwater Biol. 13:313–321.

Spacie, A. and J.L. Hamelink, 1985. Chapter 17. Bioaccumulation. In: Fundamentals of aquatic toxicology, edited by G.M. Rand and S.R. Petrocelli, Hemisphere Pub. Corp., New York, pp. 666.

Strong, C.R. and S.N. Luoma, 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.

Unlu, M.Y. and S.W. Fowler, 1979. Factors affecting the flux of arsenic through the mussel, *Mytilus gallovincialis*. Mar. Biol. 51, 209–219.

USEPA, 1978. US Environmental Protection Agency: Environmental Monitoring Series. Quality assurance guidelines for biological testing. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, EPA-600/4–78–043.

Wagner, J.G., 1975. Fundamentals of clinical pharmacokinetics. Drug Intelligence Pub., Inc., Hamilton, Illinois, 354 pp.

Watling, R.J., T.P. McClurg and R.C. Stanton, 1981. Relation between mercury concentration and size in the mako shark. Bull. Environ. Contam. Toxicol. 26, 352–358.

Whicker, F.W. and V. Schultz, 1982a. Radioecology: nuclear energy and the environment, Vol. I. CRC Press, Inc., Boca Raton, 212 pp.

Whicker, F.W. and V. Schultz, 1982b. Radioecology: nuclear energy and the environment, Vol. II. CRC Press, Inc., Boca Raton, 228 pp.

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, 213–220.

Willis, J.N. and N.Y. Jones, 1977. The use of uniform labeling with zinc-65 to measure stable zinc turnover in the mosquitofish *Gambusia affinis*. I. Retention. Health Phys. 32, 381–387.