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The Role of the Zebra Mussel, *Dreissena polymorpha*, in Contaminant Cycling: I. The Effect of Body Size and Lipid Content on the Bioconcentration of PCBs and PAHs

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ABSTRACT. *The zebra mussel,* Dreissena polymorpha, *a recent invader to the Great Lakes, may influence contaminant cycling by bioconcentrating high levels of hydrophobic contaminants in its tissue. To better understand zebra mussel bioconcentration and ultimately, contaminant cycling, we measured bioconcentration factors (BCFs) and kinetic parameters for accumulation of polychlorinated biphenyl and polycyclic aromatic hydrocarbon congeners for two size classes of mussels and for a pre-spawning (high lipid) and post-spawning (low lipid) mussel population. High lipid, pre-spawning mussels had greater BCFs and faster uptake kinetics for the highly hydrophobic compounds, i.e, hexachlorobiphenyl and benzo(a)pyrene, than the low lipid, post-spawning mussels. BCFs and uptake kinetics determined for the less hydrophobic compounds, i.e., tetrachlorobiphenyl and pyrene, were not measurably influenced by differences in lipid content. Small mussels (15 mm shell length) had higher BCFs and faster uptake kinetics for all compounds compared to larger (21 mm shell length) mussels. Contaminant elimination was not affected by size or differences in lipid levels. For both lipid levels and size classes of mussels, the BCFs were positively correlated with compound log octanol:water partition coefficient. Hence, the lipid affinity of a compound can be a good indicator of mussel contaminant accumulation. Potentially large contaminant concentration in zebra mussels may alter contaminant cycling in the Great Lakes by increasing contaminant transfer to mussel predators. Selective predation on small, pre-spawning (high lipid) mussels may present a greater hazard to predators than predation on larger, post-spawning (low lipid) mussels.*

INDEX WORDS: *Bioconcentration, zebra mussel, PCB, PAH, kinetics.*

INTRODUCTION

The zebra mussel, *Dreissena polymorpha*, is a recent invader of the North American Great Lakes ecosystem (Hebert *et al.* 1989). Zebra mussels are reported to be candidates for bioaccumulation of hydrophobic contaminants such as polychlorinated biphenyls (PCBs), chlorinated insecticides, e.g., DDT, and polycyclic aromatic hydrocarbons (PAHs) (Fisher *et al.* 1993). Their large bioaccumulation capacity, coupled with high densities in littoral areas associated with contaminated outfalls (Yankovich and Haffner 1993) and their low position in the food chain gives zebra mussels great potential to affect contaminant cycling in the Great Lakes. There are two major ways in which zebra mussels could affect contaminant cycling: 1) by increasing the contaminant concentration in sediments through biodeposition of contaminated feces and pseudofeces and; 2) by increasing trophic transfer of contaminants to mussel predators. Therefore, the

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degree to which mussels may accumulate contaminants is a primary determinant of the zebra mussel's potential impact on contaminant trophic transfer.

Contaminant accumulation in bivalves is regulated by several physiological parameters such as age, size, and lipid content (Stegeman and Teal 1973, Boyden 1974, Ernst *et al.* 1976, Cossa *et al.* 1980, Strong and Luoma 1981, Muncaster *et al.* 1990). Because lipid reserves are a primary site of nonpolar, hydrophobic xenobiotic storage, lipid content is an important factor for determining hydrophobic compound accumulation (Geyer *et al.* 1985). The high lipid content of zebra mussels has been cited as a major reason for the order of magnitude difference in bioconcentration factors (BCFs) between zebra mussels and *Mytilus edulis*, a marine mussel (Fisher *et al.* 1993). In addition, lipid levels in bivalves vary throughout the season, particularly after spawning when body lipid levels may decline by 50% (Sprung 1993). Seasonal variations in lipid levels have modified contaminant accumulation in field populations of marine bivalves (McDowell-Capuzzo *et al.* 1989, Hummel *et al.* 1990). Presently, the influence of seasonal variations in zebra mussel lipid reserves on hydrophobic contaminant accumulation is unknown.

Body size also influences contaminant accumulation in bivalves. Size-dependent accumulation of trace metals and hydrophobic contaminants such as PCBs has been reported for both freshwater and marine bivalves (Boyden 1974, Cossa *et al.* 1980, Strong and Luoma 1981, Muncaster *et al.* 1990). Smaller individuals generally have faster rates of uptake and accumulate greater contaminant concentrations than larger individuals (Cossa *et al.* 1980, Muncaster *et al.* 1990). Physiological differences in metabolic demand are the primary cause for the differential contaminant accumulation, although size-dependent feeding behavior, surface to volume ratios and concentrations of enzymes that influence uptake may also play a role (Boyden 1974, Newman and Mitz 1988).

Because of the possible influence of body size and lipid reserves on accumulation of hydrophobic xenobiotics, the effects of these variables on zebra mussel contaminant biconcentration were examined. Using kinetic methodology, BCFs and kinetic parameters for contaminant bioconcentration of two size classes of zebra mussels were determined. In addition, accumulation kinetics in pre- (high lipid) and post-spawning (low lipid) mussel populations were examined for mussels collected from the western basin of Lake Erie. The compounds studied were specific congeners from PCB and PAH chemical classes which have been identified as Great Lakes priority pollutants (Fitchko 1986). The compounds varied in lipid affinity as measured by log octanol:water partition coefficients (log K_{ow}) and thus allowed examination of the relationship between compound hydrophobicity and xenobiotic bioconcentration.

MATERIALS AND METHODS

Chemicals

^{14}C-labeled PCBs were obtained from Sigma Chemical Company (St. Louis, Missouri) and ^{3}H-PAHs were obtained from NCI Radiochemical Carcinogen Repository, Chemsyn Science Laboratories (Lenexa, Kansas). Specific activities for each chemical were: 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP), 20 mCi/mmol; 2,2′,4,4′-tetrachlorobiphenyl (TCBP), 13.8 mCi/mmol; benzo(a)pyrene (BaP), 380 mCi/mMol; and pyrene, 507 mCi/mmol. All chemicals were greater than 97% pure as determined by thin layer chromatography and radiometric analysis (Leversee *et al.* 1982). All work with PAHs was performed under gold light to minimize photodegradation.

Organisms

Zebra mussels (*Dreissena polymorpha*) were collected from littoral zones of Lake Erie by SCUBA divers from near Kelly's Island, Ohio. Mussels were scraped from rocks and transported within 3 h (in moist air) to the laboratory. Stocks of 2,000 mussels were maintained in 200-L tanks filled with filtered, aerated tap water at 20 ± 2°C and fed 150 mL of a Tetra Min (fish food) solution (10.5 g/L) per 3,000 individuals daily. Temperature, dissolved oxygen, and ammonia concentration were checked daily and water was replaced 1–2 times/week. All waste water was treated with chlorine bleach (50 ppm) before disposal to prevent introduction of veligers into inland waters. Zebra mussels were acclimated to laboratory conditions for at least 1 week prior to experimentation and only conditioned mussels were used in experiments. Zebra mussels were considered suitable for use if, after severing of byssal threads, they could reattach to a substrate within 48 h. Two size (shell length) classes of mussels, 21 ± 1 mm and 15 ± 1 mm, were selected for use.

Media

Soft (hardness 40–48 mg/L as $CaCO_3$ and total alkalinity 0.6–0.7 meq/L, 30–35 mg/L as $CaCO_3$), buffered, standard reference water (SRW) adjusted to pH 8 (USEPA 1975) was used in all experiments. Because potassium-containing compounds can be toxic to zebra mussels (Fisher *et al.* 1991), 1.0 M Na_2HPO_4 was substituted for the recommended K_2HPO_4 buffer.

Media Preparation

One day prior to initiation of uptake or elimination experiments, SRW (15 L) was spiked with radiolabeled compound dissolved in 44–284 μL of acetone and stirred for 1 h. After equilibration (4–5 h), the spiked water was divided among the glass test containers (1 L glass beakers) and the foil-covered containers were held in darkness overnight in an environmental chamber (Forma Scientific N37422, Marietta, Ohio) at 20°C. On the test day, the initial toxicant concentration was determined for each container before placing zebra mussels into the test containers. Exposure concentrations were 0.8, 0.7, 0.5, and 0.4 ppb for HCBP, TCBP, BaP and pyrene respectively.

Uptake Clearance Experiment

Uptake clearance experiments measured the rate at which mussels remove dissolved contaminants from water. Three days before an uptake experiment, individual zebra mussels were placed in 40-L aquaria filled with SRW (pH 8). The bottoms of the aquaria were covered with 2.5 × 7.5 cm glass microscope slides to which the zebra mussels reattached. The aquaria were maintained at experimental conditions and the mussels were fed daily (10 mL TetraMin solution/aquarium).

On the day of an experiment, single mussels attached to glass microscope slides were rinsed and placed in 1-L glass beakers containing 500 mL of SRW spiked with a radiolabeled PCB or PAH congener. Mussels were not fed during the exposure portion of the experiment. Zebra mussel filtration was monitored visually throughout the experiment. Mussels were assumed to be filtering if both inhalant and exhalant siphons were extended. After 0.5, 1, 1.5, 2, 4, or 6 h of exposure, five beakers were removed from the chamber. At each sampling time, three (1 mL) water samples were taken from each beaker for radiometric analysis. The zebra mussels were processed for liquid scintillation counting (LSC) (see below). To complete the mass balance analysis, the amount of compound adsorbed to the glassware was determined. Empty beakers were rinsed with clean water, then with 20 mL of acetone. A 1 mL sample of the acetone rinse was analyzed for radioactivity.

Elimination Experiment

Three days prior to an elimination experiment, zebra mussels were allowed to attach to the bottom of 100 mm glass petri dishes as described for the uptake experiments. On the day of an experiment, 100 mussels attached to 10 petri dishes were exposed to a compound for 6 h in five replicate aquaria filled with spiked SRW. The filtering of each mussel was monitored visually every hour during exposure. After 6 h, the petri dishes were removed from the spiked water and rinsed thoroughly with unlabeled SRW. Mussels that had failed to filter over the entire exposure period were removed with forceps and the remaining mussels were placed in five replicate glass elimination chambers (4 L) filled with aerated SRW. Elimination chambers were maintained in an environmental chamber at 20°C. Five zebra mussels (one mussel per elimination chamber) were removed for analysis at 12, 24, 48, 72, 96, 120, 144, and 168 h. Water in elimination chambers was changed daily after the mussels were fed (TetraMin solution). Contaminant levels in the water were monitored by taking three 1-mL water samples at each sampling period.

All mussels removed for radiometric analysis were blotted dry, weighed, and eviscerated. The rinsed valves and viscera were blotted dry, weighed, and placed in separate 20-mL glass scintillation vials with 5 mL of scintillation cocktail (1,000 mL dioxane, 500 g naphthalene, 5 g diphenyloxazole). The mussel samples were extracted for 24 h in the cocktail (extraction efficiency >90%) and then the water and mussel samples were analyzed by LSC using a Beckman LS 6000IC liquid scintillation system (^{14}C efficiency >95%; ^{3}H efficiency >60%) with automatic quench control. Contaminant concentration was determined on a wet-weight basis.

Lipid Analysis

Forty mussels from each size class were eviscerated, the tissues were blotted dry, and wet weights recorded. Tissues from 20 mussels were placed in individual test tubes and homogenized by hand in 6 mL (21 mm size class) or 3 mL (15 mm size class) of chloroform/methanol 2:1. Test tubes were sealed with aluminum foil and refrigerated overnight. A 0.5 mL sample of each mussel extract was analyzed for total lipids using the colorimetric method of van Handel (1985). Remaining mussel viscera (n = 20) were dried 24 h (56 ± 2°C) and cooled in a desiccator for 24 h before weighing. A dry to wet weight ratio was determined to convert total lipids to% dry mussel weight.

Kinetic Models

A mass-based model was used to estimate contaminant uptake clearance, k_u (Fisher *et al.* 1993). This uptake clearance describes the volume of water cleared of contaminant per mass of organism per time and has units of mL g^{-1} h^{-1}. This coefficient is conditional

with respect to the experimental parameters under which k_u is measured. In this calculation, the organism is defined as wet tissue only. Contaminants are assumed to partition between the organism and water such that;

$$dQ_a/dt = k_1Q_w - k_dQ_a. \quad (1)$$

Assuming mass balance in the system:

$$A = Q_w + Q_a \quad (2)$$

where Q_a = quantity of contaminant in the animal (μg)
k_1 = conditional uptake rate constant (h^{-1})
Q_w = quantity of contaminant in the water (μg)
k_d = the conditional elimination rate constant (h^{-1})
A = total amount of compound in the system (μg).

The basic assumptions of the model are: 1) that the mass of contaminant in the system does not change; and 2) that no biotransformation of contaminant takes place. Zebra mussel biotransformation of PAHs was examined using thin layer chromatography (TLC) and radiometric analysis (Leversee *et al.* 1982). No biotransformation was found after a 24 h exposure.

Because sorption to glassware and shells was <3% of the total mass and significant elimination did not occur over the exposure time (6 h) these parameters were eliminated from the equation. The following simplified integrated initial rates equation resulted:

$$k_1 = (-\ln(1-Q_a/A))/t \quad (3)$$

This conditional rate constant (k_1) is a system dependent value and must be converted to a system independent clearance (k_u) by the following equation;

$$k_u = k_1 \text{ (volume of water/wet mass of tissue).} \quad (4)$$

The elimination data were fit to a first order elimination model:

$$dC_a/dt = k_dC_a \quad (5)$$

Integration of equation 5 yields:

$$\ln C_a = \ln C_{ao} - k_dt \quad (6)$$

where the elimination rate constant (k_d) is determined from the slope of the regression line for $\ln C_a$ vs t.

Bioconcentration factors were calculated from the ratio of the uptake clearance and elimination rate constants:

$$BCF = k_u/k_d. \quad (7)$$

Statistical Analysis

Log transformed k_u and BCF values were analyzed by a general linear analysis of variance (GLM ANOVA, SAS 1982) and means were separated using least squares means test (GLM, SAS 1982). Significance was determined at $p < 0.05$. k_d values were tested for homogeneity of slopes (SAS 1982) and significant slopes were separated using nonoverlapping 95% confidence intervals (NCSS 1991). Individuals with uptake clearance rates <100 mL g^{-1} h^{-1} or from systems with a mass-balance <70% were not included in the data analysis.

RESULTS

Monitoring of Lipid Levels

Before we could collect mussels in the desired reproductive state, it was necessary to define the lipid levels for a pre- and post-spawning zebra mussel field population. Therefore, lipid levels of a western basin Lake Erie mussel population were determined from March through October for the 1991 and 1992 reproductive seasons (Fig. 1). Lipid levels measured in both years followed the spawning cycle of zebra mussels in the western basin (Nichols and Kollar 1991; A. Stoeckmann, pers. comm., Ohio State University, Columbus, Ohio). Lipid levels for the 21 mm size class were highest in late-May (1991) or mid-June (1992) when a high percent of mussels in the population was ripe. Lipid levels then declined over the summer as mussels spawned. Based on these data, the high lipid or pre-spawning population was defined as one in which the% lipid by dry weight was ≥9.0% and the post-spawning population was defined as having% lipid <7.0%. Mussels used in the high lipid experiments were collected in late May (1991) or mid June (1992) and post-spawning mussels were collected in early September (1991). Lipid levels were determined in cultured zebra mussels prior to experimentation. Lipid levels were 2 times greater in high lipid mussels (Mean = 13.5%) than low lipid groups (Mean = 5.9%). In both 1991 and 1992, lipid levels in the 15 mm size class were greater than 7.0% on the dates collected, thus the effect of varying lipid content on accumulation was not determined for this size class. In addition, the lipid level of the 1992, 21 mm size class did not fall below 7.0%, thus restricting these populations to the high lipid kinetic experiments.

Lipid Levels and Bioconcentration

Overall, zebra mussel contaminant bioconcentration, as measured by BCF, was high regardless of com-

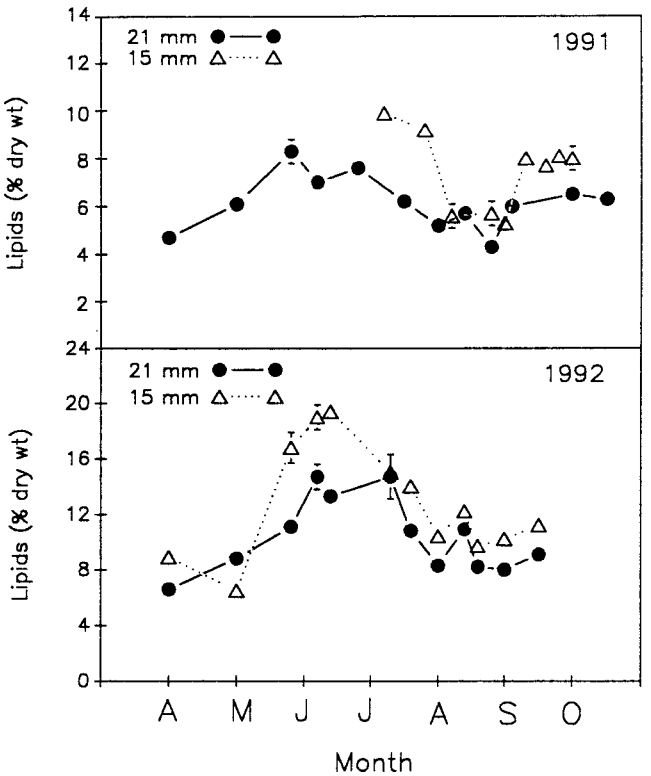

FIG. 1. Seasonal variation in lipid levels (% dry wt) of 15 and 21 mm size classes of zebra mussels collected from the western basin of Lake Erie, 1991 and 1992.

pound or lipid level (Tables 1 and 2). Uptake clearances were rapid (320 to 900 mL g^{-1} h^{-1}), while elimination was slow (k_d = 0.005 to 0.024 h^{-1})(Tables 1 and 2). In general, uptake clearances were greater and elimination rate constants were smaller for high lipid mussels (Fig. 2). However, only BCFs of the more hydrophobic compounds, HCBP and BaP, were significantly affected by changes in lipid levels (Fig. 2). This difference in contaminant bioconcentration between lipid levels for HCBP and BaP was due to a significantly greater uptake clearance in high lipid mussels. Elimination rate constants for these compounds did not vary with lipid content. Differences in lipid levels did not result in significant differences in uptake or elimination of the less hydrophobic compounds, TCBP and pyrene. Consequently, BCFs for these compounds were statistically identical. Lipid normalized BCFs indicated that the relationship of BCFs to compound lipid affinity did not change significantly within a lipid level (Tables 1 and 2).

Size and Bioconcentration

Comparison of kinetic parameters between the high lipid 15 and 21 mm mussel size classes, indicated that bioconcentration of all compounds was greater for the smaller mussels, but this effect was statistically significant only for TCBP and pyrene (Fig. 3) (Tables 1 and 3). Smaller mussels (15 mm shell length) had significantly greater uptake clearances for all four compounds than the 21 mm sized mussels. Elimination rate constants, however, were statistically identical between sizes except for BaP (Fig. 3). The effect of size on bioconcentration was not significantly altered when lipid normalized BCFs were compared.

Compound Hydrophobicity and Bioconcentration

BCFs increased with increasing compound log K_{ow} regardless of lipid level or size. In contrast, elimination rate constants decreased with increasing compound log K_{ow} (Tables 1–3). Although differences in BCFs among compounds within a size or lipid class were not always statistically different from each other, the regression of log BCF with log K_{ow} was positively correlated (Fig. 4). As with the BCFs, uptake clearances among compounds in the various size and lipid classes were not always significantly different from each other; however, the log k_u for high lipid mussels of both 21 and 15 mm size classes was positively correlated with log K_{ow} (log k_u = 0.024 log K_{ow} – 1.31 r^2 = 0.75 and log k_u = 0.25 log K_{ow} + 1.57 r^2 = 0.90 respectively). Elimination rate constants were negatively correlated with log K_{ow} for both size classes (log k_d = –0.37 log K_{ow} + 0.3, r^2 = 0.85, 21 mm; log k_d = –0.29 log K_{ow} – 0.15, r^2 = 0.91, 15 mm) and lipid levels (log k_d = –0.37 log K_{ow} + 0.3, r^2 = 0.85, high lipids; and log k_d = –0.36 log K_{ow} + 0.27, r^2 = 0.72, low lipids).

DISCUSSION

Effect of Differences in Lipid Levels on Bioconcentration

As in other organisms, the lipid reserve of bivalves is important in the accumulation and storage of hydrophobic contaminants (Hansen *et al.* 1978, Tanabe and Tatsukawa 1987). The relative size of the lipid pool affects the movement of hydrophobic chemicals into the pool such that bivalves with higher lipid levels have faster uptake and slower elimination of hydrophobic contaminants. This results in a greater total contaminant accumulation in the high lipid bivalves than in those with smaller lipid reserves (Stegeman and Teal 1973, Hansen *et al.* 1978). Consequently, the high lipid, pre-spawning zebra mussels were expected to have

TABLE 1. Kinetic parameters for the high lipid, 21 mm size class zebra mussels. Within a column, values with the same letter(s) are not significantly different. Numbers in parenthesis are standard errors.

Compound	k_u mL g^{-1} h^{-1}	k_d h^{-1}	BCF mL g^{-1}	BCF lipid normalized mL g^{-1}	Log K_{OW}	N
HCBP	900[a] (111)	0.005[a] (0.001)	173×10^3 [a] (22×10^3)	9.6×10^6 [a] (1.2×10^6)	6.90[1]	24
BaP	760[a] (91)	0.009[a] (0.001)	84×10^3 [b] (10×10^3)	4.7×10^6 [b] (0.54×10^6)	5.98[2]	25
TCBP	420[b] (43)	0.016[bc] (0.002)	27.5×10^3 [c] (2.8×10^3)	1.4×10^6 [c] (0.14×10^6)	5.90[1]	26
PYRENE	350[b] (34)	0.021[c] (0.003)	16×10^3 [d] (1.6×10^3)	0.84×10^6 [d] (0.081×10^6)	5.20[2]	26

[1]Shiu and Mackay (1986)
[2]Miller *et al.* (1985)

TABLE 2. Kinetic parameters for the low lipid, 21 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.

Compound	k_u mL g^{-1} h^{-1}	k_d h^{-1}	BCF mL g^{-1}	BCF lipid normalized mL g^{-1}	Log K_{OW}	N
HCBP	380[a] (69)	0.006[a] (0.001)	63×10^3 [a] (11.5×10^3)	7.1×10^6 [a] (1.3×10^6)	6.90	14
BaP	330[a] (68)	0.008[a] (0.001)	41×10^3 [a] (8.5×10^3)	4.6×10^6 [ab] (0.93×10^6)	5.98	11
TCBP	440[a] (52)	0.021[b] (0.001)	21×10^3 [b] (2.5×10^3)	2.9×10^6 [b] (0.34×10^6)	5.90	18
PYRENE	320[a] (34)	0.024[b] (0.002)	13×10^3 [c] (1.3×10^3)	1.8×10^6 [cb] (0.19×10^6)	5.20	18

TABLE 3. Kinetic parameters for the high lipid, 15 mm size class zebra mussels. Numbers in parenthesis are standard errors. Within a column, values with the same letter(s) are not significantly different.

Compound	k_u mL g^{-1} h^{-1}	k_d h^{-1}	BCF mL g^{-1}	BCF lipid normalized mL g^{-1}	Log K_{OW}	N
HCBP	1,680[a] (215)	0.007[a] (0.001)	240×10^3 [a] (31×10^3)	9.1×10^6 [a] (1.2×10^6)	6.90	25
BaP	1,300[ab] (186)	0.017[b] (0.002)	77×10^3 [b] (11×10^3)	3.1×10^6 [b] (0.45×10^6)	5.98	25
TCBP	990[b] (118)	0.014[b] (0.002)	71×10^3 [b] (8.5×10^3)	2.9×10^6 [b] (0.35×10^6)	5.90	27
PYRENE	640[c] (73)	0.021[b] (0.004)	35×10^3 [c] (4.8×10^3)	1.9×10^6 [c] (0.57×10^6)	5.20	27

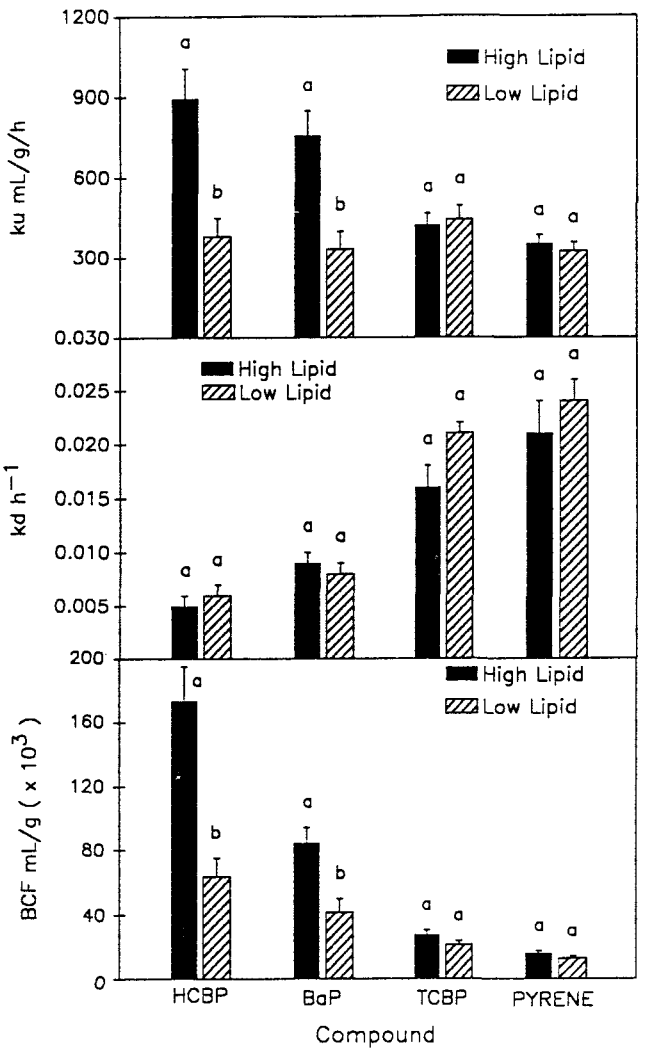

FIG. 2. Comparison of kinetic parameters between the high and low lipid levels, 21 mm size class zebra mussels. For each compound, values with the same letter are not significantly different.

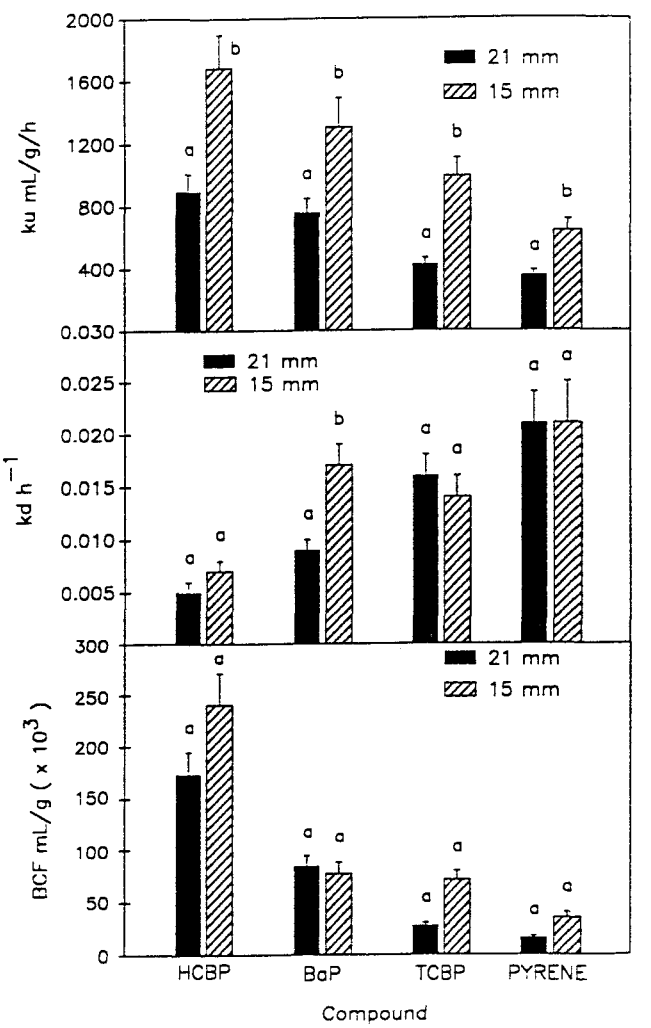

FIG. 3. Comparison of kinetic parameters between the 21 and 15 mm size class, high lipid level zebra mussels. For each compound, values with the same letter are not significantly different.

greater contaminant bioconcentration as measured by BCFs and it was expected that their bioconcentration kinetics would follow the kinetics described above for other bivalves. As predicted, pre-spawning zebra mussels had significantly greater bioconcentration of the more hydrophobic compounds than post-spawning mussels (Fig. 2). High lipid mussels also exhibited faster uptake of these two compounds relative to the low lipid, post-spawning mussels. Lipid normalized BCFs for HCBP and BaP were statistically identical for both the high and low lipid mussels suggesting that the differential accumulation between the pre- and post-spawning mussels resulted from differences in lipid pool size.

Contrary to expectations, HCBP and BaP elimination rate constants were not significantly different between high and low lipid mussels; thus, uptake rather than elimination mechanisms appear to drive accumulation in high lipid animals. The lack of differences in elimination rate constants between the high and low lipid mussels argues against the lipid storage theory in which high lipid mussels experience lower elimination. A more probable explanation is that the lipid pool of the mussels was not completely saturated by contaminants, a condition that is necessary to see alteration of k_d due to differences in size of lipid pools (Bickel 1984). The short exposure time, prior to elimination,

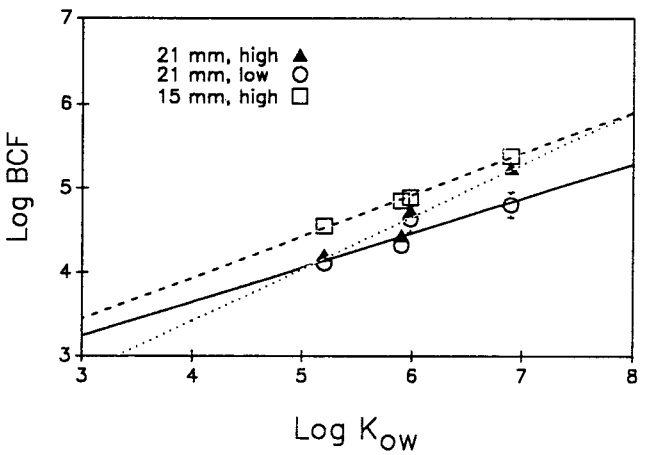

FIG. 4. Log BCF vs log K_{ow} for 21 and 15 mm, high lipid mussels and 21 mm low lipid mussels. (Log BCF = 0.62 Log K_{ow} + 0.95, r^2 = 0.94, p <0.05, 21 mm, high lipid) (Log BCF = 0.41 Log K_{ow} + 2.02, r^2 = 0.85, p < 0.05, 21 mm, low lipid) (Log BCF = 0.49 Log K_{ow} + 1.97, r^2 = 0.99, p < 0.05, 15 mm, high lipid).

ensured that lipid saturation did not occur even in low lipid, post-spawning mussels.

Differences in HCBP and BaP uptake clearances also could be due to differences in condition between the two groups rather than differences in the lipid pool size. For instance, seasonal reduction in food availability caused a slowing of metabolic rates in zebra mussels collected in late summer from Lake St. Clair as compared to metabolic rates measured for mussels collected in spring when food was plentiful (Quigley *et al.* 1993). Post-spawning mussels used in the kinetic experiments were collected in late summer. It is possible that feeding and length of the acclimation period prior to experimentation may not have been sufficient to allow the mussels to recover from reduced food stores. Hence, lower filtration rates due to a seasonal reduction in food could account for reduced uptake of HCPB and BaP in the post-spawning population.

Differences in lipid levels between the two groups did not affect bioconcentration of less hydrophobic TCBP and pyrene as defined by BCFs. In addition, both TCBP and pyrene were accumulated to a lesser extent that the more hydrophobic HCBP and BaP. A number of studies have reported that low chlorinated biphenyls and lower molecular weight PAHs are not retained in bivalves as readily as are the more hydrophobic HCBP and BaP and therefore their accumulation is not as readily influenced by lipid content (Neely *et al.* 1974, Vreeland 1974, Geyer *et al.* 1982, McDowell-Capuzzo *et al.* 1989). Thus, the physicochemical properties of TCBP and pyrene may account for the lack of significant difference in their accumulation kinetics and bioconcentration between the pre- and post spawning groups.

Effect of Size on Bioconcentration

Field studies have shown that contaminant concentrations in mussels and body size are negatively correlated (Boyden 1974, Cossa *et al.* 1980, Muncaster *et al.* 1990, Fisher *et al.* 1993). Size-dependent contaminant bioconcentration is attributed to a link between metabolic rates and rates of uptake or loss of contaminant; smaller individuals compensate for higher metabolic demands by increasing their respiration (filtering) rates thereby increasing their exposure to water-borne contaminants (Boyden 1974, Muncaster *et al.* 1990). Contaminant uptake rates are reported to be greater in smaller individuals for a wide range of aquatic organisms (Anderson and Spear 1980, Newman and Mitz 1988), and for some invertebrates they have been positively correlated with oxygen clearance rates (Landrum *et al.* 1992). In this study, contaminant uptake clearances were significantly higher for 15 mm zebra mussels for all compounds tested. There is a twofold increase in oxygen consumption for 15 mm vs 21 mm zebra mussels (Fisher *et al.* 1993) suggesting that contaminant uptake is linked to the greater oxygen demands of 15 mm mussels. However, Fisher *et al.* (1993) reported that high filtration rates in zebra mussels reflect food requirements rather than oxygen demand. Since filtration rates in mussels are inversely related to size (Vahl 1973, Bayne and Widdows 1978), the size-dependent uptake of waterborne contaminants in zebra mussels is more likely related to nutrient requirements than oxygen demands. Because both size classes were pre-spawning mussels, the lipids levels and condition (Mean = 13.5 and 15.5% dry wt respectively) of the classes were similar and did not contribute to differences in uptake. The similarity in elimination rate constants between the two size classes supports the argument that size-dependent uptake of contaminants drives bioconcentration.

Correlation of Compound Lipophilicity with Bioconcentration

The positive correlation between compound lipid affinity (K_{ow}) and an organism's BCF is highly predictive of hydrophobic contaminant bioconcentration (Geyer *et al.* 1982,1985; Hawker and Connell 1986). Significant correlations were found for the regression of log K_{ow} with log k_u and BCFs for both size classes of pre-spawning mussels and with the BCFs of 21 mm

post-spawning mussels. Similar positive correlation of log K_{ow} values for PCBs and PAHs with uptake clearances or BCFs have been reported for zebra mussels (Fisher *et al.* 1993) and the blue mussel, *Mytilus edulis* (Pruell *et al.* 1986). Hence, compound lipid affinity appears to accurately predict mussel accumulation of highly hydrophobic contaminants such as PCBs and PAHs. The absence of a correlation of compound log K_{ow} with uptake clearances or elimination rate constants in post-spawning zebra mussels may be due to their poor condition rather than hydrophobicity of the chemicals tested. Thus, the predictive value of K_{ow} may be modified by physiological changes in condition due to reproductive state. The negative correlation of lipid affinity with elimination rate constants found in both the 21 and 15 mm size classes of mussels confirms similar results reported for the zebra mussel (Fisher *et al.* 1993) and for the green lipped mussel (Tanabe and Tatsukawa 1987).

Ecotoxicological Perspective

Zebra mussels in both size classes and lipid levels significantly accumulated hydrophobic, non-polar contaminants. If waterborne contaminants are the primary source of contaminant exposure, uptake will be rapid and bioconcentration will result in significant concentrations of these contaminants in the zebra mussels. If these contaminated mussels are ingested by zebra mussel predators including fish and water fowl, the contaminants could then move up through aquatic food chains. In the case of predatory waterfowl, deleterious effects of zebra mussel-directed contaminant uptake may extend to the northern breeding areas of the Great Lakes (de Kock and Bowmer 1993). Recent reductions in breeding success of herring gulls and bald eagles which feed in Lake Erie have been attributed to zebra mussel-directed trophic transfer of PCBs (J. Reuter, pers. comm., Ohio State University, Columbus, Ohio).

Smaller mussels with high lipid levels had the greatest bioconcentration of contaminants. Therefore, selective predation on small, pre-spawning (high lipid) mussels may present a greater hazard to aquatic or terrestrial predators than predation on larger, post-spawning (low lipid) mussels. However, mussels in both size classes and lipid levels were able to bioconcentrate significant levels of contaminants. Consequently, contaminant transfer from a wide size range of mussels to predators can occur over an entire season.

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REFERENCES

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

Bayne, B.L., and Widdows, J. 1978. The physiological ecology of two populations of *Mytilus edulis* L. *Oecologia* (Berlin) 37:137–162.

Bickel, H. 1984. The role of adipose tissue in the distribution and storage of drugs. *Prog. Drug Res.* 28:237–303.

Boyden, C.R. 1974. Trace elements content and body size in molluscs. *Nature* 251:311–314.

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

de Kock, W. Chr., and Bowmer, C.T. 1993. Bioaccumulation, biological effects and food chain transfer of contaminants in the zebra mussel (*Dreissena polymorpha*). In *Zebra Mussels: Biology, Impacts, and Control.* eds. T.F. Nalepa and D.W. Schloesser, pp. 503– 533. Chelsea, Mich.: Lewis Publishers.

Ernst, W., Goerke, H., Eder, G., and Schaefer, R.G. 1976. Residues of chlorinated hydrocarbons in marine organisms in relation to size and ecological parameters. *Bull. Environ. Contam. Tox.* 15:55–65.

Fisher, S.W., Strongberg, P., Bruner, K.A., and Boulet, L.D. 1991. Molluscicidal activity of potassium to the zebra mussel, *Dreissena polymorpha*: toxicity and mode of action. *Aqua. Tox.* 20:219–234.

_____, Gossiaux, D.C., Bruner, K.A., and Landrum, P.F. 1993. Preliminary investigations of the toxicokinetics of hydrophobic contaminants in the zebra mussel, *Dreissena polymorpha* Pallas. In *Zebra Mussels: Biology, Impacts, and Control*, eds. T.F. Nalepa and D.W. Schloesser, pp. 465–490. Chelsea, Mich.: Lewis Publishers.

Fitchko, J. 1986. *Literature review of the effects of persistent substances in Great Lakes biota.* Report of the health of aquatic communities task force, Windsor, Ontario.

Geyer, H., Sheehan, P., Kotzias, D., Freitag, D., and Korte, F. 1982. Prediction of ecotoxicological behavior of chemicals: relationship between physico-chemical properties and bioaccumulation of organic chemicals in the mussel *Mytilus edulis*. *Chemosphere* 11: 1121–1134.

_____, Scheunert and Korte, F.. 1985. Relationship between the lipid content of fish and their bioconcentration potential of 1,2,4-trichlorobenzene. *Chemosphere* 14:545–555.

Hansen, N., Jensen, V.B., Appelquist, H., and Morch, E. 1978. The uptake and release of petroleum hydrocarbons by the marine mussel *Mytilus edulis*. *Prog. Wat. Tech.* 10:351–359.

Hawker, D.W., and Connell, D.W. 1986. Bioconcentration of lipophilic compounds by some aquatic organisms. *Ecotoxicol. Envir. Saf.* 11:184–197.

Hebert, P.N.D., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.* 46:1587–1591.

Hummel, H., Bogaards, R.H., Nieuwenhuize, J., DeWolfe, L., and Van Liere, J.M. 1990. Spatial and seasonal differences in the PCB content of the mussel *Mytilus edulis*. *Sci. Total. Environ.* 92:155–163.

Landrum, P. F., Frez, W.A., and Simmons, M.S. 1992. Relationship of toxicokinetic parameters to respiration rates in *Mysis relicta*. *J. Great Lakes Res.* 18:331–339.

Leversee, G.J., Geisy, J.P., Landrum, P.F., Gerould, S., Bowling, J.W., Fannin, T.E., Haddock, J.D., and Bartell, S.M. 1982. Kinetics and biotransformation of benzo(a)pyrene in *Chironomus riparius*. *Arch. Environ. Contam. Toxicol.* 11:25–31.

McDowell-Capuzzo, J.M., Farrington, J.W., Rantamaki, P., Clifford, C.H., Lancaster, B.A., Leavitt, D.F., and Jia, X. 1989. The relationship between lipid composition and seasonal differences in the distribution of PCBs in *Mytilus edulis* L. *Mar. Environ. Res.* 28:259– 264.

Miller, M.M., Wasik, S.P., Huang, G., Shiu, W., and Mackay, D. 1985. Relationships between octanol-water partition coefficient and aqueous solubility. *Environ. Sci. Technol.* 19:522–529.

Muncaster, B.W., Hebert, P.D.N., and Lazar, R. 1990. Biological and physical factors affecting the body burden of organic contaminants in freshwater mussels. *Arch. Environ. Contam. Toxicol.* 19:25–34.

NCSS. 1991. Number Crunching Statistical Software. Kaysville, Utah.

Neely, W.B., Branson, D.R., and Blau, G.E. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. *Environ. Sci. Technol.* 8: 1113–1115.

Newman, M.C., and Mitz, S.V. 1988. Size dependence of zinc elimination and uptake from water by mosquitofish *Gambusia affinis* (Baird and Girard). *Aqua. Tox.* 12:17–32.

Nichols, S.J., and Kollar, B. 1991. Reproductive cycle of zebra mussels (*Dreissena polymorpha*) in western Lake Erie at Monroe, Michigan. Conference Proceeding of the Second International Zebra Mussel Research Conference, Rochester, New York.

O'Neill, C.R., and MacNeil, D.B. 1989. *Dreissena polymorpha*: an unwelcome new Great Lakes invader. NR Sea Grant Cooperative Extension. Cornell Univ., Ithaca, NY.

Pruell, R.J., Lake, J.L., Davis, W.R., and Quinn, J.G. 1986. Uptake and depuration of organic contaminants by blue mussels (*Mytilus edulis*) exposed to environmentally contaminated sediment. *Mar. Biol.* 91:497– 507.

Quigley, M.A., Gardner, W.S., and Gordon, W.M. 1993. Metabolism of the zebra mussel (*Dreissena polymorpha*) in Lake St. Clair of the Great Lakes. In *Zebra Mussels: Biology, Impacts and Control*, eds. T.F. Nalepa and D.W. Schloesser, pp. 295–306, Chelsea, Mich.: Lewis Publishers.

SAS. 1982. *SAS User's Guide*. SAS Institute Inc., Cary, North Carolina.

Shiu, W.Y., and Mackay, D. 1986. A critical review of aqueous solubilities, vapor pressures, Henry's law constants, and octanol-water partition coefficients of the polychlorinated biphenyls. *J. Phys. Chem. Ref. Data* 15:911–929.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. In *Zebra Mussels: Biology, Impacts and Control*, eds. T.F. Nalepa and D.W. Schloesser, pp. 39–54, Chelsea, Mich.: Lewis Publishers.

Stegeman, J.J., and Teal, J.M. 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster *Crassostrea virginica*. *Mar. Biol.* 44:37–44.

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

Tanabe, S., and Tatsukawa, R. 1987. Mussels as bioindicators of PCB pollution: A case study on uptake and release of PCB isomers and congeners in green-lipped mussels (*Perna viridis*) in Hong Kong waters. *Environ. Poll.* 47:41–62.

USEPA. 1975. *Methods for acute toxicity testing with fish, macroinvertebrates and amphibians*. EPA-6601/3-75-009. National Technical Information Service, Springfield, Va.

Vahl, O. 1973. Pumping and oxygen consumption rates of *Mytilus edulis* L. of different sizes. *Ophelia* 12:45–52.

van Handel, E. 1985. Rapid determination of total lipids in mosquitoes. *J. Am. Mosq. Control Assoc.* 1:302–304.

Vreeland, V. 1974. Uptake of chlorobiphenyls by oysters. *Environ. Pollut.* 6:135–140.

Yankovich, T.L., and Haffner, G.D. 1993. Habitat selectivity by the zebra mussel (*Dreissena polymorpha*) on artificial substrates in the Detroit River. In *Zebra Mussels: Biology, Impacts, and Control*, eds. T. F. Nalepa and D.W. Schloesser, pp. 175–184, Chelsea, Mich.: Lewis Publishers.

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