J. Great Lakes Res. 22(2):379–388
Internat. Assoc. Great Lakes Res., 1996

Effect of Temperature on the Accumulation Kinetics of PAHs and PCBs in the Zebra Mussel, *Dreissena polymorpha**

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ABSTRACT. *The role of temperature on the accumulation and elimination kinetics of selected polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCB) congeners was examined for the zebra mussel,* Dreissena polymorpha. *Uptake and elimination rates were measured at intervals over a three year period in laboratory toxicokinetics studies in which zebra mussels were maintained at ambient field temperatures or acclimated to higher or lower temperatures. The uptake rate coefficients (k_u) for benzo(a)pyrene (BaP) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP) increased in proportion to temperature over a range from 4 to 20°C when measured at the field-collected temperature. Both k_u and the elimination coefficient (k_d) also decreased with increasing organism size. However, no relationship with temperature was found for the k_u of BaP in the following year nor was there a relationship between k_u and temperature for pyrene and pentachlorophenol (PCP) when the kinetics were measured at field-collected temperatures. In laboratory acclimation studies, k_u for three compounds (BaP, HCBP, and PCP) showed a significant positive relationship with temperature over the 4–20°C range. Pyrene accumulation kinetics however, still exhibited no relationship with temperature. These results contradict the concept that uptake of contaminants changes over a season with temperature thereby, influencing contaminant toxicokinetics. Furthermore, the k_d values observed for these compounds indicate that elimination was slow, and no relationship of k_d was exhibited with water temperature, season, or year.*

INDEX WORDS: *PAHs, PCBs, zebra mussels, temperature, kinetics.*

INTRODUCTION

Since its invasion of Lake Erie in 1988, the zebra mussel, *Dreissena polymorpha*, has been under investigation for its role in contaminant cycling (O'Neill and MacNeill 1989). Of the contaminants impacting Lake Erie, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) constitute two of the major groups of concern because of their detectable presence in water, biota, sediment, and suspended particles (Fitchko 1986). Although the effects of zebra mussels on contaminant cycling have not been thoroughly studied, zebra mussels are able to accumulate PCBs and other organic compounds not only from the water but from contaminated solids such as algae and sediment (Roper and Cherry 1994; Bruner *et al.* 1994 a,b; Fisher *et al.* 1993). Because of their high filtration rates and production of feces and pseudofeces, zebra mussels may cause an enhanced transport of contaminants from the pelagic to the benthic environment with a concomitant effect on the bioavailability of organic compounds in the ecosystem (Bruner *et al.* 1994b).

The role of temperature in the contaminant cycling process may be very important because low water temperatures slow the filtration process, inhibit growth, and may expand the life expectancy of the zebra mussel (Reeders *et al.* 1989, Dorgelo and Gorter 1984, Stanczykowska 1977). Preliminary results from laboratory temperature controlled studies for dissolved benzo(a)pyrene (BaP) and hexachlorobiphenyl (HCBP) demonstrated that both the uptake rate coefficient (k_u) and elimination rate constant (k_d) increased with temperature increases

*GLERL Contribution # 957.

(Fisher *et al.* 1993). However, various other studies demonstrated conflicting findings for filter feeding bivalves, including zebra mussels. Algal filtering rates decreased on either side of an optimum temperature of 12.5–15.0°C (Waltz 1978). Also, filtering rates dropped sharply at temperatures under 5°C and above 20°C. (Reeders and Bij deVaate 1990). Finally, filtering rates increased linearly with increased temperature from 8–25°C (Stancyzykowska 1977). Similar conflicting findings in filtering rates have also been observed with the marine mollusc *Mytilus edulis* (Theede 1963, Schulte 1975, Ali 1970). Although preliminary data suggested a strong thermal response in toxicokinetic parameters with laboratory thermal acclimation, we attempted to verify the thermal acclimation by comparing the toxicokinetics of laboratory-acclimated zebra mussels to those collected and maintained at field temperatures. Our primary objective was to examine the adaptation of the thermal response in the toxicokinetics through short-term water-only exposures.

MATERIALS AND METHODS

Organisms

Adult zebra mussels were collected from Lake St. Clair (42°20′00″ N and 82°47′30″ W) at a water depth of 5 m. They were collected using an epibenthic sled, cleaned with lake water, placed in a cooler, covered with wet paper towels, and transported to the laboratory. At the laboratory, mussels were transferred to aerated aquaria and maintained at the same water temperature at which the mussels were collected. Mussels in culture were fed a daily diet of algae consisting of *Chlorella* granules, manufactured by Sun Chlorella Inc., and *Chlamydomonus* spp. The *Chlorella* was prepared by adding 3 g of dried algae to 100 mL of distilled water. The slurry was sonicated to break up clumps, and frozen. The frozen cubes were suspended over the aquaria and allowed to melt. The *Chlamydomonus* was prepared in a stock solution using Guillard WC culture medium (Guillard and Lorenzen 1972).

Mussels used in these studies had a shell length of 14-22 mm. The lipid content of 10 individual zebra mussels was determined from each collection time to monitor organism health. The lipids were measured using a microgravimetric procedure with chloroform/methanol extraction (Gardner *et al.* 1985).

Chemicals

All radioisotopes were purchased from the Sigma Chemical Company (St. Louis, MO). The compounds studied included ^{3}H-benzo(a)pyrene (BaP, 33.1 Ci/mMol), ^{14}C-BaP (26.6 mCi/mMol), ^{3}H-pyrene (34 Ci/mMol), ^{14}C-pentachlorophenol (PCP, 7.9 mCi/mMol), and ^{14}C-2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP, 17.6 mCi/mMol). All compounds were dissolved in an acetone carrier and when administered to exposure water ranged between 0.005 mL/L to 0.01 mL/L. Compound radiopurity was > 98% for all compounds. The radiopurity was determined by thin layer chromatography (TLC), using hexane:benzene (8:2, v:v) as the solvent system for BaP, HCBP, and pyrene, and bezene:ethyl acetate (3:1 v:v) for PCP. The radiopurity was quantified by liquid scintillation counting (LSC). Analytical procedures were performed under gold fluorescent light (λ > 500 nm) to minimize the photodegradation of the PAHs.

Toxicokinetic Studies

Toxicokinetic studies with zebra mussels consisted of aqueous exposures to radiolabled compounds performed in filtered lake water maintained at the field collectiion temperature. All exposures were performed in temperature controlled environmental chambers. Studies were generally carried out within the first 72 h after the collection was made. The mussels were not fed during any portion of the uptake study but were fed daily as described above with *Chlorella* during the elimination phase. Studies were also performed where mussels were exposed to radiolabled compounds after being acclimated to temperatures higher or lower than the ambient field temperatures. For the acclimation studies, the mussels were acclimated by either increasing or decreasing the water temperature 2°C per day from the collection temperature. Mussels were held at the experimental temperatures for a minimum of 24 h prior to use. Aquarium water was monitored biweekly for ammonia and replaced weekly. All acclimation experiments were carried out between 10 and 22 days after collection. Three acclimation studies were conducted with mussels collected in spring (March 1992) and fall (November 1990 and 1991) where mussels were acclimated from 4–15°C. One study was conducted with animals collected in summer (June 1993) in which mussels were acclimated from 15-4°C.

Filtered lake water (12 L) adjusted to the experimental temperature, was dosed in bulk with dual-labeled compounds in the following combinations: BaP/HCBP, BaP/PCP, BaP/pyrene. In each study one compound was ^{3}H-labeled and the other was ^{14}C-labeled. However in two cases, water was

dosed with only a single label, either PCP, or BaP. The water was allowed to equilibrate for 1 h after dosing. 500 mL of dosed water was then poured into 21, 600-mL beakers, and placed into an environmental chamber at the experimental temperature. Mussels were considered suitable for use if, after their removal from the attached substrate, they could reattached to petri-dishes within 48 h. The animal and petri-dish was submerged, one animal per beaker with three water only beakers for controls. Initial water concentrations (time 0) were then determined. Animals from replicate beakers (n = 3) were removed and sampled at 0.5, 1, 1.5, 2, 3, and 6 h. Contaminant concentration was determined for water, shell and tissue using LSC.

For LSC, tissue samples were dissected, weighed, and placed in 12 mL of scintillation cocktail (RPI 3a70B). Each tissue was then intensely agitated for 1 min. using a Tekmar 375-watt ultrasonic processor (Tekmar Co., Cincinnati, OH). Shells were placed in scintillation cocktail without sonication for 24 h and then removed before counting. Sorption to the beaker was also determined by measuring the radioactivity in an acetone rinse of each beaker. Mass balance was maintained within the system with the total amount of radioactivity added to the system remaining constant over the time-course of the experiment. The total accountability at the end of the exposures ranged from 85 to 93%. The ^{14}C and ^{3}H tracers were counted simultaneously on a LKB 1217 liquid scintillation counter using dual-label counting (counting efficiency = 91 ± 1% for ^{14}C and 62 ± 1% for ^{3}H). The data were corrected for quench using external-standards ratio method after correcting for background,.

Elimination of contaminants was determined by transferring contaminated mussels to clean uncontaminated aerated water after 6 h of simultaneous exposure with mussels used in the uptake studies. The rates of elimination were determined by sampling over a 15 d period post-exposure. During this time, half the water was changed every third day to avoid buildup of ammonia and to decrease the potential recycling of test compound that may have been released by the mussels. The mussels were fed a daily diet of *Chlamydomonus* spp. Contaminant concentrations for tissue and shell were determined in triplicate for each of the six sample times (same times varied for each experiment performed).

The four laboratory adjusted temperature studies were performed using the same procedure as above, except that mussels were acclimated to test temperatures several days before analysis.

Kinetics

Accumulation was modeled through a mass balance model: Equation 1 (Landrum *et al.* 1992). Using the initial rates assumptions, the model assumed that during the uptake phase elimination was not significant, no biotransformation of contaminants occurred, and the total mass of contaminants remained the same throughout the exposure. The amount of compound sorbed onto the shell and to the beaker were found to be small and assumed to remain constant over the course of the 6 h exposure and were, therefore, not incorporated into the mass balance. Also, biotransformation potential of the mussels was examined by tissue extraction and followed by TLC (Fisher *et al.* 1993). Using this procedure the mussels exhibited no evidence of contaminant biotransformation after 6 h of exposure.

$$k_1 = \frac{-\ln\left(1 - \frac{Q_a}{A}\right)}{t} \quad (1)$$

Q_a = mass of compound in the organism (g)
A = total mass of compound in the water at the beginning of the exposure (g)
t = time (h)

k_1 is a conditional rate constant which is a system dependent value and must be converted to a system independent clearance (k_u) by the following equation (Landrum *et al.* 1992)

$$k_u = k_1 \text{ (Volume of water/Wet mass of tissue)} \quad (2)$$

The uptake clearance, k_u, then describes the amount of water scavenged of contaminant per amount of tissue per time (mL g^{-1} -wet tissue h^{-1}).

The elimination rate coefficient, k_d, was determined using a first order elimination model.

$$C_a^l / C_a^o = e^{-k_d * t} \quad (3)$$

C^l_a = concentration in the animal at anytime t (g g^{-1})
C_a^o = concentration in the animal at time zero (g g^{-1})

Bioconcentration factors (BCF, L Kg^{-1}) were calculated from uptake and elimination rate constants:

$$BCF = k_u / k_d \quad (4)$$

Elimination half-lives ($t_{1/2}$) were calculated directly from measured k_d values (h):

$$t_{1/2} = 0.693/k_d \quad (5)$$

Statistics

Multiple regressions, students t-test, and analysis of variance were conducted on various toxicokinetic, environmental, and biological parameters such as uptake rate coefficient, temperature, and organism size using Systat. (Wilkinson 1992). The significance level was set at $p < 0.05$.

RESULTS

Seasonal Variation Studies

Masses of shell and sorption to beakers ranged from 0.2 to 2.0 % and was subsequently not included into the uptake model. In uptake studies performed on zebra mussels at the *in situ* temperature, contaminant accumulation was rapid for mussels collected at sampling dates from fall 1990 to spring 1993 (Tables 1 and 2) with the exception of mussels collected in March 1992. The animals obtained in March 1992 were collected at 4°C water and appeared to be under considerable stress from overwintering. These mussels exhibited very slow uptake. Visual examination revealed that the mussels would only remain open for a total of 1 h during a 6 h study, which probably contributed to the slow uptake rate.

The uptake rate coefficients measured for BaP at different collection times in the 1990 seasonal studies were statistically different from one season to the next using a students t-test ($p < 0.05$, $n = 40$). However, there was no relationship between collection date and k_u for BaP in 1991. Furthermore, an investigation of BaP k_u values from 1990 to 1993 reveals a significant increase over this time period, ANOVA $p < 0.05$, $n = 166$.

HCBP uptake clearance exhibited a significant increase with increasing temperature and decreasing zebra mussel mass as determined by regression analysis $k_u = 34.6$ (± 7.9 S.E.) temperature (°C) - 4.7 (± 1.5) wet weight (g) + 757.8 (± 153); $r^2 = 0.387$, $p < 0.001$, $n = 46$. This trend was calculated using data from two years (Table 2).

Data analysis revealed that uptake rate coefficients for pyrene tested at any water temperature were not statistically different from one another over the 3-year period. Pyrene k_u values ranged from 427 mL g^{-1} h^{-1} in 1990 to 512 mL g^{-1} h^{-1} in 1993 for water temperatures within 6°C of each other (Table 2).

In a season-to-season study in 1991, PCP showed no significant difference in k_u within a season at different temperatures ranging from 4–24°C.

Elimination

The analyses of k_d and BCF for BaP, pyrene and PCP showed no consistent trends in the data for either water temperature, season, or year (Tables 1 and 2). For HCBP, there was a positive relationship for k_d with temperature and a negative relationship for BCF with temperature. However, there are a limited number of sampling points and it is uncertain whether the trend will continue. Earlier work by Fisher *et al.* (1993) did reveal a positive change in elimination rate constant as a function of temperature and negative change in log octanol:water partition coefficient.

Use of the described methodology in the elimination studies raised a question of potential recycling of eliminated contaminants due to infrequent water exchange. However both by calculation and experiment this issue was examined. The calculations indicated that maximum error in the data would be about 20% for the 24 h time point assuming no sorption or other loss processes for the highly hydrophobic contaminants. In an experimental design that directly compared the original design and a design that had water exhange after 12 h, 24 h, and every 48 h thereafter, the data for the elimination by the two groups completely overlapped and no effect of recycling of the contaminants BaP and HCBP could be observed.

Acclimation Studies

In studies that used animals acclimated to temperatures different than *in situ* temperatures, uptake coefficients (k_u) for BaP, HCBP, and PCP increased as temperature increased:

$$k_{uBaP'91} = 36.9\ (\pm 8.9)\ T + 363.7\ (\pm 93.5),\quad r^2 = 0.271,\ p < 0.005;$$

$$k_{uHCBP} = 10.5\ (\pm 6.6)\ T + 553.7\ (\pm 84.1),\quad r^2 = 0.716,\ p = 0.358$$

$$k_{uBaP'90} = 9.3\ (\pm 1.8)\ T + 370.8\ (\pm 24.6),\quad r^2 = 0.964,\ p = 0.121$$

$$k_{uPCP} = 9.1\ (\pm 0.5)\ T + 22.4\ (\pm 5.1),\quad r^2 = 0.997,\ p = 0.03.$$

TABLE 1. BaP toxicokinetics from ambient field temperature water only exposure.

Collection Date	Collection Temperature °C	Lipid % ± S.D.	k_u mL $g^{-1}h^{-1}$ ± S.D.	Samples n =	k_d h^{-1} ± S.E.	BCF	$t_{1/2}$	T_o Water Concentration ng/L
25/7/90	20	9 ± 2	995 ± 323	12	0.009 ± 0.0003	77,000	77	4.83–5.28
23/8/90	23	8 ± 2	688 ± 269	11	0.016 ± 0.0004	49,000	42	3.65–3.76
15/11/90	4	8 ± 2	415 ± 149	17	0.002 ± 0.0006	191,000	330	5.80–6.39
2/5/91	8	nm[1]	646 ± 239	17	0.003 ± 0.0009	167,000	231	6.86–9.59
14/5/91	18	15 ± 2	443 ± 249	16	0.003 ± 0.0015	132,000	223	2.70–3.15
1/7/91	24	7 ± 2	692 ± 386	15	0.003 ± 0.0005	165,000	223	2.37–3.19
1/7/91	24	7 ± 2	998 ± 490	16	0.004 ± 0.0006	150,000	157	2.54–3.07
21/11/91	4	7 ± 2	592 ± 203	15	0.003 ± 0.0002	197,000	231	1.81–1.95
31/3/92	4	10 ± 2	28 ± 20	16	0.001 ± 0.0001	40,000	693	67.1–79.4
28/4/92	10	13 ± 4	1,364 ± 436	15	0.005 ± 0.0004	24,000	154	2.45–2.89
10/6/93	15	nm	1,113 ± 641	16	0.005 ± 0.0013	273,000	138	2.60–3.21

[1] = not measured
[2] = not able to calculate value
[3] -collection temperature = exposure temperature

TABLE 2. HCBP, PCP, and pyrene toxicokinetics from ambient field temperature water only exposure.

Collection Date	Collection Temperature °C	Lipid % ± S.D.	k_u mL $g^{-1}h^{-1}$ ± S.D.	Samples n =	k_d h^{-1} ± S.E.	BCF	$t_{1/2}$	T_o Water Concentration ng/L
HCBP								
25/7/90	20	9 ± 2	1102 ± 356	12	0.004 ± 0.0001	178000	173	2.71-3.01
15/11/90	4	8 ± 2	564 ± 214	17	0.001 ± 0.0004	550000	693	2.19-2.35
2/5/91	8	nm[1]	959 ± 288	17	0.002 ± 0.0011	506000	439	1.54-1.94
PCP								
14/5/91	18	15 ± 2	95 ± 45	12	0.006 ± 0.0008	10000	119	2.60-3.07
1/7/91	24	7 ± 2	110 ± 73	10	0.005 ± 0.0007	12000	133	2.41-2.78
1/10/91	14	7 ± 3	120 ± 47	15	0.008 ± 0.0001	14000	87	2.81-3.28
21/11/91	4	7 ± 2	135 ± 35	15	0.003 ± 0.0003	45000	231	2.06-2.26
Pyrene								
11/10/90	21	8 ± 3	427 ± 209	15	0.009 ± 0.0001	33000	72	3.05-3.23
31/3/92	4	10 ± 2	43 ± 22	15	0.002 ± 0.0001	22000	346	9.84-10.24
28/4/92	10	13 ± 4	687 ± 292	15	0.006 ± 0.0002	77000	123	0.74-2.22
10/6/93	15	nm	512 ± 199	16	0.008 ± 0.0009	39000	86	0.40-0.49

[1] = not measured
[2] = not able to calculate value

(values in parenthesis are ± S.E.)(Tables 3, 4). Also, for BaP, k_u decreased linearly as mussels were acclimated to decreasing temperature,

$$k_u = 60.5\ (\pm\ 15.6)\ T + 228\ (\pm\ 169),$$
$$r^2 = 0.257,\ n = 45.$$

However, unlike HCBP, there was no improvement in the relationship when tissue mass was factored into the linear regression (Table 3).

For pyrene, no change in k_u was observed in studies using animals acclimated to either higher or lower temperature than those at the time of collection. The uptake rate coefficients, k_u, for pyrene in mussels tested from 15–4°C ranged from 512 mL g^{-1} h^{-1} at 15°C to 340 mL g^{-1} h^{-1} at 4°C (Table 5).

TABLE 3. BaP toxicokinetics from laboratory temperature acclimation exposures.

Collection Date	Exposure Temperature °C	k_u mL g^{-1} h^{-1} ± S.D.	k_d h^{-1} ± S.E.	BCF	$t_{1/2}$ h
15/11/90[1]	4	415 ± 149	0.002 ± 0.0006	190,000	330
	12	514 ± 225	0.006 ± 0.0005	83,000	115
	20	882 ± 403	0.009 ± 0.0005	61,000	77
21/11/91[2]	4	592 ± 203	0.003 ± 0.0002	197,000	231
	10	545 ± 217	0.003 ± 0.0005	220,000	231
	15	1,028 ± 310	0.007 ± 0.0009	116,000	99
31/3/92[3]	4	28 ± 20	0.001 ± 0.0001	40,000	693
	10	1,008 ± 660	0.005 ± 0.0003	147,000	133
	15	1,364 ±1,151	0.004 ± 0.0011	215,000	173
10/6/93[6]	15	1,113 ± 641	0.005 ± 0.0013	270,000	138
	10	870 ± 332	0.009 ± 0.0022	107,000	77
	4	449 ± 337	0.009 ± 0.0066	62,000	77

[1] Acclimation Study from 4 to 20°C
[2] Acclimation study from 4 to 15°C
[3] Acclimation Study from 4 to 15°C[4] Acclimation study from 15 to 4°C

TABLE 4. HCBP and PCP toxicokinetics from laboratory temperature acclimation exposure.

Collection Date	Exposure Temperature °C	k_u mL g^{-1} h^{-1} ± S.D.	k_d h^{-1} ± S.E.	BCF	$t_{1/2}$ h
HCBP 15/11/90[1]	4	564 ± 214	0.001 ± 0.0004	550,000	693
	12	715 ± 280	0.004 ± 0.0001	175,000	138
	20	1,048 ± 459	0.004 ± 0.0007	188,000	173
PCP 21/11/91[2]	4	135 ± 35	0.003 ± 0.0004	45,000	231
	10	134 ± 44	0.004 ± 0.0004	28,000	173
	15	247 ± 93	0.007 ± 0.0004	23,000	99

[1] Acclimation Study from 4 to 20°C
[2] Acclimation study from 4 to 15°C

TABLE 5. Pyrene toxicokinetics from laboratory acclimation exposure.

Collection Date	Exposure Temperature °C	k_u mL g^{-1} h^{-1} ± S.D.	k_d h^{-1} ± S.E.	BCF	$t_{1/2}$ h
31/3/92[3]	4	43 ± 22	0.002 ± 0.0001	32,000	346
	10	645 ± 304	0.009 ± 0.0004	48,000	77
	15	664 ± 454	0.009 ± 0.0007	41,000	77
	15	512 ± 199	0.008 ± 0.0009	39,000	86
	10	493 ± 242	0.013 ± 0.0011	24,000	53
	4	340 ± 182	0.008 ± 0.0030	39,000	87

Also, elimination rate coefficients were similar in each test, averaging 0.0094 ± 0.0021 h^{-1}, n = 5.

DISCUSSION

Seasonal Variation Studies

Based on previous toxicokinetic studies on zebra mussels (Fisher *et al.* 1993), k_u and k_d increased for BaP and HCBP with an increasing temperature from 4-24°C in laboratory acclimation. This resulted in a significant decrease in $t_{1/2}$ with increasing temperatures. However, when mussels were tested at the temperature of collection, the data did not show these same trends for BaP possibly due to a larger, more significant data set. When the uptake rate coefficients were examined for BaP in 1991, the variability in these values increased, resulting in no significant difference between the tested temperature and k_u throughout the year. Furthermore, since there was no observed change in k_u, we hypothesize that the mussels optimize their filtering rate at a high level under field conditions. In contrast, mussels held in the laboratory can be induced to show a temperature response resulting in an increase in bioaccumulation.

Metabolic activity of the zebra mussels varies widely between spring, summer, and fall, as measured by oxygen (O_2) consumption and nitrogen (N) excretion (Quigley *et al.* 1993). Highest O_2 consumption occurs in spring and early summer (32–45 mgO_2 g^{-1} -dry weight d^{-1}) and is significantly lower in the summer and fall. When O_2 consumption was measured in the laboratory across a temperature range, a similar Q_{10} (the change in O_2 consumption for every 10°C in temperature) of 2.14 (Fisher *et al.* 1993) was observed compared to that observed for the field. Thus, k_u should also vary with season if it is driven by the respiration process, but this was not observed. Overall, the metabolic rate may have a minimal effect on contaminant accumulation by the zebra mussel but rather the filtering rate for food (algae) may be the driving force governing contaminant accumulation.

Quigley *et al.* (1993) also reported no differences in respiration rates of zebra mussels exposed at 10 and 20°C for smaller-sized animals (shell lengths 5–16 mm), while O_2 consumption doubled from 10–20 °C for mussels of shell length 24–25 mm. Shell length of mussels in this study were 14–22 mm, which may have been too narrow a size range for any significant metabolic increase (and k_u increase) to be observed with rising temperature. Further, Quigley *et al.* (1993) stated that food deprivation may depress metabolic rates, and the effect might be greatest in smaller animals due to their higher metabolic rates and lower energy reserves. This may have been the cause of the slow uptake rate exhibited by the over-wintered animals (March 1992 collection).

The constant uptake rate observed by zebra mussels from water temperatures between 5–20°C is not unusual. Reeders *et al.* (1989) noticed that filtration activity of *D. polymorpha* does not significantly change over this range of water temperatures. However, at temperatures lower than 5°C and greater than 20°C, filtration rates decline significantly. Thus, our experimental temperature range may have been too limited to detect changes in animals tested at the temperature of field collection. But the over-wintered mussels would have been stressed due to the lower temperatures. This probably explains the unusually low filtering rates for these animals.

As previously observed by Reeders *et al.* (1989), there was no significant change in uptake rate within a season. However, there was a statistically significant change between seasons. Our mussels collected at 4°C in March 1993 had virtually no measurable clearance rate. Calculating uptake rate coefficients on an individual basis (Eq. 1), k_u values of 28 (± 20 S.D.) mL g^{-1} h^{-1} for BaP and 43 (± 22 S.D.) mL g^{-1} h^{-1} for pyrene were obtained. The difference in k_u constants from this 4°C field temperature study compared with November 1990 and November 1991's 4°C studies may be the result of the use of mussels that were collected at different metabolic stages. There was also a significant difference in BaP uptake rates from year to year in mussels tested at field temperatures. The reason for the difference has not been resolved. However, there may be a decline in gross primary productivity in the water column due to increasing water quality changes such as those observed in western Lake Erie during 1988 to 1990 where there was a 43% decline in mean chlorophyll *a* concentrations and an 85% increase in Secchi disc transparencies (Leach 1993). Such a decline could yield food limitations and create a resultant stress. Another possible reason for the difference in uptake rates, maybe due to the stress the organism is experiencing in the field when low algae concentrations are measured in the water. These low concentrations of food resources in turn deplete the lipid reserves thus making the organism unresponsive to the thermal stress. When the animals are fed in the laboratory prior to

the acclimation studies, the food source may increase the organism's health thus allowing to better adjust to thermal changes. Food quality may be a third reason in the uptake differences. The food source in the laboratory has changed to a more natural food selection, *Chlamydomonus*, which may be found in the field, as compared to the dead *Chlorella*.

Although accumulation was rapid, the zebra mussels did not reach steady-state based on calculated BCFs comparing C_a/C_w, at the end of the experiment, to k_u/k_d. There is a factor greater than 100 between the values. This is apparently different from observations with *Mytilus edulis*, another small mussel, where accumulation of naphthalene was rapid and approached steady-state within 4 h (Widdows *et al.* 1983). While it was possible to achieve steady-state with PAHs, such as naphthalene, having low log K_{ow} in short time periods, the difference in lipid concentrations between the two species may indicate why zebra mussels come to steady state more slowly. *M. edulis* has less than 2% lipids by dry weight (Renberg *et al.* 1986), and *D. polymorpha* range from 7–15% lipids depending upon the season in which they were analyzed.

A positive correlation between organism shell size and k_u has been described for zebra mussels at 20°C with lipid concentrations > 9% (Brunner *et al.* 1994 a). Although mussels were not intended to be separated into two distinctive size classes for this study, the k_u constants calculated in the 14–17 mm size class were generally greater than those in the 18–22 mm size class (Fig. 1). However, due to the small sample size, the mean value for each class has a large standard deviation that produces non-statistically significant differences between the size classes.

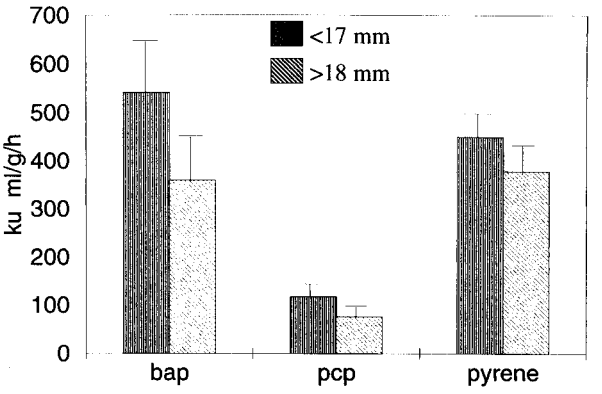

FIG. 1. Uptake rate constant between mussels less then 17 mm and greater then 18 mm. For each compound there is no statistical difference between the two size classes.

Elimination

The elimination half-life for these compounds was slow relative to that observed for a fish of the same weight (Spacie *et al.* 1983). Slow elimination has also been observed for *Mytilus edulis* and other bivalves when exposed to PAHs and PCBs in field studies or in sediment-dosed experiments (Kannan *et al.* 1989, Pruell *et al.* 1986). In water-only exposures, black mussels, *Mytilus galloprovinciallis*, exposed to various concentrations of water soluble fractions of Qatar light crude oil also showed slow elimination rates (Mason 1988). In short-term exposures, 72–144 h, *M. galloprovinciallis* exhibited a $t_{1/2}$ that was less than 120 h. Also, half-life estimates in long-term exposures, 46 and 95 d, resulted in $t_{1/2}$ of 16–29 d. It is uncertain whether similar trends in the data would be found for *D. polymorpha* if longer exposures were explored. Animal lipid content, exposure concentration, and exposure route would play a factor in half life determination. Widdows *et al.* (1983) showed that *Mytilus edulis* with relatively high lipid levels in tissue eliminated over 90% of total naphthalene taken up in tissues within 192 h. In contrast, tissues with lower lipid levels eliminated naphthalene at a slower rate. The combined elimination results in a biphasic elimination. This pattern has also been seen for *Mytilus* for a variety of other PAHs and PCBs (Broman and Ganning 1986, Hansen *et al.* 1978). In this study, only monophasic elimination could be observed. However, there were slight differences in elimination of compounds with higher log K_{ow}. BaP and HCBP exhibited little loss over the first 24 h (Fig. 2). Thereafter, the concentrations in the mussels' tissue decreased rapidly over the next 48–168 h. Elimination of the more water-soluble compounds, pyrene and PCP, occurred rapidly over the first 24 h then slowed over the remainder of the elimination period (Fig. 3). This monophasic elimination has been described by other investigators who have found that depuration rates depend on lipophilicity of the chemical (Bruner *et al.* 1994a, Clark and Findley 1975, Pruell *et al.* 1986, Lee *et al.* 1972, Dunn and Stich 1976, Hawker and Connel 1986) and lipid content of the organism (Landrum 1988).

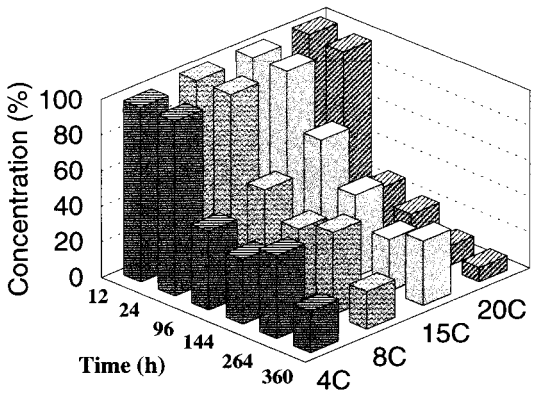

FIG. 2. BaP elimination as percent change in concentration in zebra muscles at each of the study temperatures.

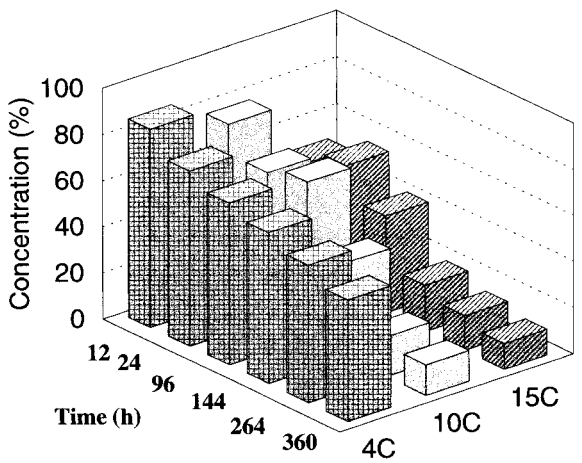

FIG. 3. Pyrene elimination as percent change in concentration in zebra muscles at each of the study temperatures.

CONCLUSION

There was no evidence to suggest that uptake of contaminants by *D. polymorpha* changes over the course of a season due to water temperature as observed from 4-24°C. However, it is possible that during the winter months, with water temperatures of 4°C or below, zebra mussels become incapable of filtering water due to a reduction in metabolism brought about by the extreme cold. When mussels are tested at the temperature of field collection, k_u doesn't change suggesting that the mussels optimize their filtering rate to a high level under field conditions. In contrast, mussels held in the laboratory can be induced to show a temperature response resulting in an increase in bioaccumulation. What effect this has on contaminant cycling is still unclear. The data do show that uptake rates can change from year to year with varying environmental conditions, which presumably affect the zebra mussels physiology.

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Submitted: 14 June 1995
Accepted: 22 March 1996