

Temporal variation of organochlorine contaminants in the zebra mussel *Dreissena polymorpha* in Lake Erie

Susan L. Roe, Hugh J. MacIsaac *

*Department of Biological Sciences and Great Lake Institute for Environmental Research,
University of Windsor, Windsor, Ont. N9B 3P4, Canada*

Received 20 September 1996; received in revised form 2 July 1997; accepted 25 July 1997

Abstract

Zebra mussels (*Dreissena polymorpha*) are commonly employed as contaminant biomonitors in Europe and North America. Accumulation of hydrophobic, organochlorine contaminants into an organism depends, in part, on the organism's lipid content. In this study, we assess the influence of temporal variation in lipid content and reproductive activity on contaminant dynamics in zebra mussels collected from the western basin of Lake Erie. Mussels spawned twice during 1995, first in June and again in August. Lipid content of mussels was highest following the first spawning event and declined thereafter. Lipid-adjusted concentrations of moderately hydrophobic (mid- K_{ow}) compounds were much higher than those of either low or high hydrophobicity. Principal component analysis (PCA) of seasonal variation of concentrations of nine compounds revealed two primary axes, the first determined by mid- and high- K_{ow} compounds (PCBs 52, 101, 118, 153, 138 and 180), the latter by a low K_{ow} compound (hexachlorobenzene). A second principal component model separated physiological, environmental and temporal factors. Variation in mid- and high- K_{ow} compounds corresponded with precipitation and reproductive status of mussels. By contrast, variation in hexachlorobenzene concentration was associated with lipid content and reproductive cycle. Concentrations of all nine compounds did not differ significantly between gravid and laboratory-spawned mussels, indicating that the effect of reproduction may be decoupled from the actual spawning event. Concentrations did not differ between spent male and female mussels. Examination of lipid composition revealed that neutral lipids comprise only 39% of total lipids in zebra mussels. Concentrations of PCBs with $\log K_{ow} > 5.71$ were more highly correlated with neutral than with total lipid content, indicating that concentration normalization on a neutral lipid basis may be more appropriate than adjustment based on total lipid. Results from this study indicate that reproductive status and lipid content

* Corresponding author. Tel.: +1 519 2534243, ext. 2734; fax: +1 519 9713609; e-mail: hughm@uwindsor.ca

affect contaminant dynamics in zebra mussels and that these factors require consideration when mussels are deployed as biomonitors. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Zebra mussel; Biomonitor; Organochlorine contaminants; PCBs; Lipid; Reproduction; Great Lakes

1. Introduction

Polychlorinated organic contaminants are typically present in extremely low concentrations in lake and river water, but some readily accumulate in biota. Because of this property, biomonitors have been used successfully to identify and quantify polychlorinated organic contaminants in aquatic environments (Goldberg et al., 1978; Phillips, 1980; Duursma et al., 1984; Claisse, 1989). Mussels are particularly useful biomonitors owing to their large size, sedentary nature, long life and suspension feeding mode.

Zebra mussels have been used extensively in Europe as biomonitors of heavy metal and organochlorine contaminant exposure (Duursma et al., 1984; Kraak et al., 1991; Giese and Krüger, 1992; Mersch et al., 1992). Owing to their hydrophobic nature, most organochlorine compounds readily partition into the lipid fraction of aquatic biota. *Dreissena polymorpha* has a relatively high lipid content, 9–15% of dry weight, making it especially suitable to monitor trace organic contaminants (Mersch et al., 1992; Fisher et al., 1993; Bruner et al., 1994). Zebra mussels bioaccumulate organic contaminants up to one order of magnitude higher than other bivalves in the Great Lakes (Brieger and Hunter, 1993; Fisher et al., 1993). As the North American distribution of the zebra mussel has expanded to include most river systems in temperate eastern North America, so too has its use as a biomonitor.

Equilibrium partitioning is the principal mechanism used to explain the differential accumulation of hydrophobic chemicals into lipid compartments of aquatic biota (Mackay, 1982; Tanabe et al., 1987; Hummel et al., 1989, 1990; Secor et al., 1993). Among the assumptions implicit to this theory are that the system is in equilibrium and that lipid content remains constant over time (Connell, 1988). However, the lipid pool in bivalves fluctuates seasonally. For example, Bruner et al. (1994) demonstrated that lipid concentrations in zebra mussels in Lake Erie were highest during June and July and lowest during spring and autumn and suggested that these patterns resulted from reproductive activity.

Considering that the reproductive effort in *D. polymorpha* is high, comprising up to 45% of annual production (Sprung, 1995), reproductive activities may impact lipid and, consequently, contaminant dynamics. For example, if lipid levels change faster than contaminant dynamics can accommodate, then non-equilibrium or quasi-state conditions may persist. Previous research has measured lipid content and contaminant concentrations only a few times a year and/or sampling purposefully avoided the reproductive season. By conducting concurrent analyses of mussel reproductive status, lipid content and composition and relevant environmental

factors, we assess the role of reproductive development and spawning activity in altering contaminant body burdens.

2. Materials and methods

2.1. Study site

Zebra mussels were collected from a chemically contaminated site in western Lake Erie 1 km offshore from the Fermi Nuclear Plant in southeastern Michigan (Fig. 1). The site is ~ 7 m deep and covered with a dolomite bedrock bottom. This site is directly downstream from the Detroit River outflow, from which it receives municipal and industrial waste discharges. These discharges are contaminated with a variety of complex hydrophobic organic compounds and heavy metals (Oliver and Bourbonniere, 1985; Furlong et al., 1988). PCB concentrations in sediment near the study site average 365 ng g^{-1} (Oliver and Bourbonniere, 1985; Furlong et al., 1988). Polychlorinated biphenyls (PCBs) are made available to biota at the site via re-suspension of contaminated sediment (Fallon and Horvath, 1985).

2.2. Sample collection and preparation

Mussels were gathered usually every 10–14 days between May and October 1995, for a total of 14 collections. On each date, three replicates of several hundred mussels each were obtained by towing a benthic D-net trawl along the lake bottom. Three consecutive 300 m tows, each representing one replicate, were taken running south to north, parallel to the Michigan shoreline (Fig. 1). The starting point for the first tow was located via a global positioning system (Garmin). Mussels were thoroughly rinsed in lake water and placed in coolers containing lake water for transport to the lab.

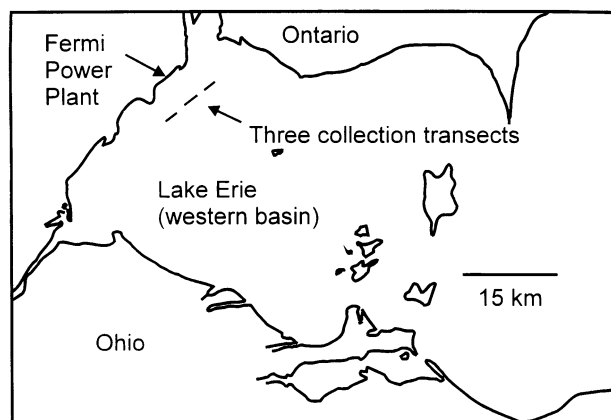


Fig. 1. Location of *D. polymorpha* sampling site in western Lake Erie. Transect lengths are not to scale.

The bottom temperature, Secchi disk depth and water depth were measured on each sampling date. Bulk surface water samples were collected in 20 l carboys for use in laboratory aquaria. Water samples for chlorophyll *a* analysis were taken with a modified Schindler–Patalis trap lowered to approximately 0.5 m above the lake bottom. Water was emptied into 1 l amber bottles. Samples for chlorophyll *a* analysis were filtered onto acetate filters (0.8- μm ; Gelman Sciences), which were then dissolved in 20 ml of 90% acetone, and stabilized with magnesium carbonate. Samples were extracted in the dark overnight at 5°C, centrifuged and absorbance measured with a narrow band spectrophotometer according to Lind (1979). Precipitation data was obtained later from the Michigan Department of Agriculture/Michigan State University Climatological Services, East Lansing, MI. Daily totals for ten stations along the Detroit River were averaged. The average daily rainfall between sampling dates was then calculated.

A random sub-sample of 15–20 mm mussels from each replicate was immediately wrapped in hexane-rinsed foil and frozen at -20°C until lipid and contaminant analysis could be performed. Remaining mussels were transferred to three 40-l aerated, glass aquaria containing lake water collected and glass-fibre filtered (Whatman 0.7- μm retention) that day. Mussels were maintained in a cold room at 12–14°C, with replicates in separate aquaria.

On each date, 30 mussels from each replicate were dissected and microscopically examined to determine status of reproductive maturity (Fisher et al., 1993). Female mussels were scored on a scale of 0–4 reflecting their reproductive state: 0 = spent; 1 = immature with sexes indistinguishable; 2 = immature with sexes differentiated; 3 = less than 50% oocytes mature; and 4 = greater than 50% of oocytes mature. Although male mussels were counted, they were not scored as to their reproductive development. Sex ratio was calculated only on dates when the sex of all mussels could be ascertained (i.e. all mussels greater than or equal to stage 2). For the purpose of calculating a mean reproductive state of females, sex ratio was used to adjust the number of mussels at stages 0 and 1 to include females only for all other samples. Mean reproductive state of females for each replicate was calculated as:

$$R = \frac{\sum_{i=1}^n V_i}{n}$$

where R is the mean reproductive state, V is the reproductive score and n is the number of female mussels examined. The relationship between date and reproductive state of females was examined through a two-way χ^2 -test of association.

Whenever possible, the diameter of ten randomly-selected oocytes from each female were measured using a squash mount preparation of the gonad sac and an ocular micrometer (Nichols, 1993). Mean oocyte diameter per replicate was calculated using mean egg diameters of each female in the replicate. Analysis of variance (ANOVA) was used to test the effect of date on mean oocyte size (i.e. experimental unit; Hurlbert and White (1993)).

2.3. Mussel contaminant and lipid analyses

Replicate samples of mussels from 11 of the 14 collection dates and laboratory-spawned male and females mussels (see Section 2.4) were selected for organic chemical analysis. Sample preparation and contaminant analysis followed techniques of Lazar et al. (1992) and IUPAC nomenclature. Of the 49 chemicals quantified in zebra mussel soft tissues, nine contaminants were selected to examine the effects of time, physiological status (i.e. mussel lipid content and reproductive status) and environmental (i.e. temperature, chlorophyll *a*, Secchi depth and precipitation) factors on chemical body burdens. Chemicals were chosen based on a complete temporal profile, relevance to published literature and representative low- (HCB (hexachlorobenzene), PCB 31/28 and PCB 52), mid- (PCB 101, PCB 118 and PCB 138) and high- K_{ow} compounds (PCB 153, PCB 180 and PCB 206). A principal component analysis (PCA) was performed on $\log_{10}(x + 1)$ wet-mass concentrations of the nine selected compounds using varimax rotation (Wilkinson et al., 1992; Tabachnick and Fidell, 1996).

Concurrent with contaminant extraction, total lipid content of soft mussel tissue was quantified gravimetrically (Lazar et al., 1992). Percent lipid content was arcsine (square root proportion) transformed to improve normality.

A second PCA was conducted to reduce dimensionality of physiological (reproductive status, gravimetric lipid concentration), environmental (Secchi depth, chlorophyll *a* concentration, water temperature and precipitation) and temporal (Julian date and reproductive cycle) parameters. Factor values from both PCAs were used in a multivariate regression model to examine the relationship between chemical principal components (dependent factors) and physiological, environmental and temporal principal components (independent factors).

Three replicates of each of sexually immature, gravid and spent mussels for each of the two reproductive cycles were selected for lipid class analysis. Total lipid was first extracted following Kates (1986). Extract (1 ml) was placed in a 2 ml glass vial secured with a Teflon-lined cap and frozen at -20°C until lipid separation. Separation of the lipid classes was performed using an Iatroscan equipped with a flame ionization detector (Parrish, 1987). Percent neutral lipid content (wet-mass basis) was calculated by summing percents hydrocarbon, wax/sterol esters, methyl ester, triacylglycerol, free fatty acids, free aliphatic alcohol, free sterol and diacylglycerol. Percent polar lipid content (wet-mass basis) was calculated by summing percents acetone-mobile polar lipids and phospholipids. Temporal variation of neutral lipids and polar lipids was assessed with a multiple analysis of variance (MANOVA). The first factor was reproductive cycle (two spawning cycles), while the second factor was reproductive status (immature, gravid and spent). Lipid concentrations were arcsine (square root proportion) transformed to normalize data prior to analysis.

Implications of different lipid adjustments to contaminant concentrations were assessed for the selected group of chemicals. $\log_{10}(x + 1)$ wet-mass chemical concentrations were correlated with the arcsine (square root proportion) concentrations of each of gravimetric total lipid (TLG), Iatroscan total lipid (TLI) and Iatroscan neutral lipid (NLI).

2.4. Effects of spawning

To isolate the effect of spawning activity on contaminant body burdens, gravid mussels were induced to spawn in the laboratory. Following each mussel collection, a trial was conducted to determine whether mussels were competent to spawn. This procedure involved placement of twenty 15–20 mm mussels into individual 20-ml glass scintillation vials containing 9 ml of filtered lake water. Mussels were allowed to acclimate for 1 h at 23–25°C before adding 1 ml of 10^{-2} M 5-hydroxytryptamine creatine sulphate salt (serotonin; Sigma) to induce spawning (Ram et al., 1993). At least 4 h was allotted for a response (Ram et al., 1993). If more than 50% of the mussels spawned during the trial, 500 mussels from each replicate (20 June and 20 July) or 100 mussels from each replicate (11 August) were similarly induced. After spawning, female and male mussels were kept separate, wrapped in hexane rinsed foil and frozen at -20°C . Three to five spawned mussels were dissected and examined microscopically to determine if spawning had been complete. The effect of spawning activity was assessed by comparing percent lipid and chemical concentrations between gravid mussels and mussels spawned in the lab. Percent lipid and lipid-adjusted contaminant concentrations were analyzed separately. Paired *t*-tests with adjusted α -values were used to test the hypotheses that there were no significant differences in either percent lipid content or lipid-adjusted concentrations of the nine selected contaminants between gravid and spent mussels (Sokal and Rohlf, 1995).

3. Results

Water temperature, Secchi disc depth and chlorophyll *a* followed trends typically published for the area (Table 1; Leach (1993), Garton and Haag (1993)). Lake temperature increased from 11°C on 12 May to a maximum of 30°C on 11 August, after which it declined to 15°C by 4 October. Chlorophyll *a* concentration peaked on 20 June at 8.4 mg m^{-3} and again on 11 August at 8.2 mg m^{-3} . Very low chlorophyll *a* concentrations were detected ($< 1 \text{ mg m}^{-3}$) on 12 May or after 21 August. Secchi depth averaged 2.68 ± 0.13 m, though no seasonal pattern was evident. Water depth was consistently 7 m.

The western basin of Lake Erie was subjected to a number of severe storms throughout the summer, though not reflected in precipitation results presented in Table 1. The most severe storm occurred on 15 and 16 July when a daily average of 28.8 and 24.2 mm of rain, respectively, was recorded over the ten selected stations. Other events occurred on 24 May (24.1 mm), 28 May (19.4 mm) and 3 October (16.4 mm).

The reproductive status of female mussels varied significantly over the summer season ($P < 0.0001$; χ^2 -test). Zebra mussels spawned twice during the sampling period; the first spawning cycle began in mid-June and continued until early July, while the second cycle occurred during August (Fig. 2). Female mussels dominated the population on 13 June and 1 August, comprising $65.6 \pm 1.1\%$ (S.E.M) and

Table 1
Physical conditions in western Lake Erie, adjacent to the Fermi nuclear power plant (Monroe, MI) during 1995

Date	Bottom temperature (°C)	Secchi depth (m)	Chlorophyll <i>a</i> (mg m ⁻³)	Precipitation (mm)
May 12	11	2.5	n.d.	3.4
May 25	15	3.8	1.6	2.7
June 06	20	2.5	1.1	2.2
June 13	18.6	4.0	0.4	0.2
June 20	23	1.5	8.4	0.0
July 03	22	2.0	1.5	4.7
July 20	25	2.8	4.9	4.3
August 01	27	2.5	8.2	4.3
August 11	30	2.2	4.2	3.0
August 21	26	2.5	2.9	4.1
September 01	23	4.0	n.d.	0.1
September 11	19.5	3.0	n.d.	0.9
September 21	17.5	2.5	n.d.	0.7
October 04	15.0	3.0	n.d.	1.9

Precipitation is the average daily rainfall between sampling dates. The value for 12 May is the average daily rainfall between 1 and 11 May.
n.d., Not detected.

$61.1 \pm 1.1\%$ (S.E.M.) of the individuals collected on these dates, respectively. Mature oocytes were observed in females from 25 May through 11 August. Mean oocyte diameter ranged from 54.8 to 87.9 μm , though it did not vary significantly by date ($P > 0.05$; ANOVA).

Lipid-adjusted concentrations of the nine selected contaminants varied little between 12 May and 3 July (Table 2). With the exception of HCB and PCB 206, contaminant concentrations increased 33–60% on 20 July relative to the previous sampling date. Concentrations then decreased slightly until 21 August, when a second, smaller increase occurred. Again, concentrations declined thereafter until the end of the study period. Concentrations were highest for PCBs with moderately high K_{ow} values and with 40–60% biphenyl chlorination. For example, PCB 138 and PCB 153 had the highest concentrations, while PCB 206 and HCB had the lowest (Table 2).

The first two principal components explained 52.0 and 15.9% of variation in the chemical data set, respectively. Principal component (PC) one was determined primarily by mid- and high- K_{ow} compounds (PCBs 101, 52, 138, 118, 153 and 180), whereas, PC 2 was determined by HCB. PC 3 and PC 4 accounted for additional 14.7 and 13.7% of variability in the chemical data and were, respectively, determined by PCB 31/28 and PCB 206.

A second principal component analysis was conducted to assess variation in independent variables. This analysis identified four primary components. PC 1 (41.4% of variance) was associated with lipid content and reproductive cycle of mussels, and Julian date. Limnological data (chlorophyll *a*, Secchi depth and

Table 2
 Temporal variation in mean (\pm S.E.M.) concentrations ($\mu\text{g kg}^{-1}$ lipid) of representative low-, mid- and high- K_{ow} compounds. Total PCBs is the sum of 39 congeners. % Lipid was determined gravimetrically and is based on wet weight of soft tissue. Only six of the nine selected contaminants are presented here

Date	% Lipid	Low- K_{ow}			Mid- K_{ow}			High- K_{ow}			Total PCBs
		HCB	PCB 31/28	PCB 101	PCB 138	PCB 153	PCB 180				
12 May	1.3 \pm 0.4	25.7 \pm 7.7	54.2 \pm 18.3	339.4 \pm 94.0	590.3 \pm 163.7	481.2 \pm 134.0	348.2 \pm 94.8	5683.5 \pm 1511.6			
6 June	1.6 \pm 0.1	33.0 \pm 5.6	76.6 \pm 7.0	338.1 \pm 23.1	559.3 \pm 49.7	473.5 \pm 20.5	327.3 \pm 29.7	5774.0 \pm 426.7			
13 June	1.4 \pm 0.0	27.2 \pm 1.0	69.0 \pm 5.2	324.3 \pm 18.1	538.0 \pm 46.2	442.2 \pm 21.5	308.6 \pm 13.5	5367.3 \pm 318.8			
20 June	1.5 \pm 0.2	29.2 \pm 1.6	86.3 \pm 4.5	345.2 \pm 18.2	603.4 \pm 40.7	469.7 \pm 29.8	337.8 \pm 18.2	6163.6 \pm 342.7			
3 July	1.4 \pm 0.1	25.7 \pm 3.0	73.6 \pm 1.9	357.9 \pm 21.1	573.6 \pm 21.9	472.9 \pm 22.7	307.5 \pm 18.2	5852.6 \pm 299.4			
20 July	1.3 \pm 0.1	19.1 \pm 0.3	90.5 \pm 7.2	605.8 \pm 7.7	916.3 \pm 5.8	714.9 \pm 12.1	507.6 \pm 5.1	9397.4 \pm 79.2			
1 August	0.9 \pm 0.1	20.1 \pm 2.2	98.3 \pm 2.3	588.7 \pm 27.5	819.3 \pm 17.0	674.5 \pm 12.6	448.0 \pm 12.8	8539.6 \pm 239.3			
11 August	0.9 \pm 0.2	35.5 \pm 6.4	100.2 \pm 12.2	526.6 \pm 88.8	817.9 \pm 137.1	663.7 \pm 117.6	437.7 \pm 69.9	8057.3 \pm 1266.4			
21 August	0.6 \pm 0.1	41.3 \pm 6.5	152.0 \pm 5.8	620.1 \pm 73.9	1018.2 \pm 166.2	861.7 \pm 103.0	544.8 \pm 68.4	10465.7 \pm 1302.6			
1 September	0.6 \pm 0.1	32.7 \pm 3.6	164.7 \pm 27.7	575.3 \pm 50.6	1003.8 \pm 114.4	828.0 \pm 92.7	572.6 \pm 51.5	9468.8 \pm 959.1			
4 October	0.6 \pm 0.1	23.3 \pm 0.6	70.7 \pm 9.7	468.9 \pm 88.5	798.6 \pm 135.2	668.4 \pm 103.0	429.4 \pm 52.1	7508.7 \pm 1305.9			

bottom temperature) was represented by PC 2 (28.8%), while precipitation determined PC 3 (13.3%). PC 4 (8.9%) was determined by mussel reproductive state. Multiple regression analysis revealed that moderately high- K_{ow} PCBs (PC 1 of chemical data set) were associated with precipitation (Wilks' $\lambda = 7.3$; $P < 0.001$) and reproductive state (Wilks' $\lambda = 4.6$; $P < 0.05$), whereas, low- K_{ow} compounds (HCB) correlated with lipid content, date and reproductive cycle (Wilks' $\lambda = 16.9$; $P < 0.001$). Neither high- nor low- K_{ow} compounds were correlated to limnological factors (Wilks' $\lambda = 0.5$; $P > 0.5$).

Introspect analysis revealed high levels of free fatty acids in mussel soft tissues, indicating the presence of oxidized lipid. Examination of individual lipid classes was, therefore, deemed inappropriate, though lipids were pooled into polar and neutral groups (Table 3). Both spawning cycle and reproductive status significantly influenced neutral lipid content ($P < 0.05$; MANOVA) but not polar lipid content ($P > 0.05$). The effect of reproductive status on neutral lipid content was also dependent on spawning cycle ($P < 0.05$).

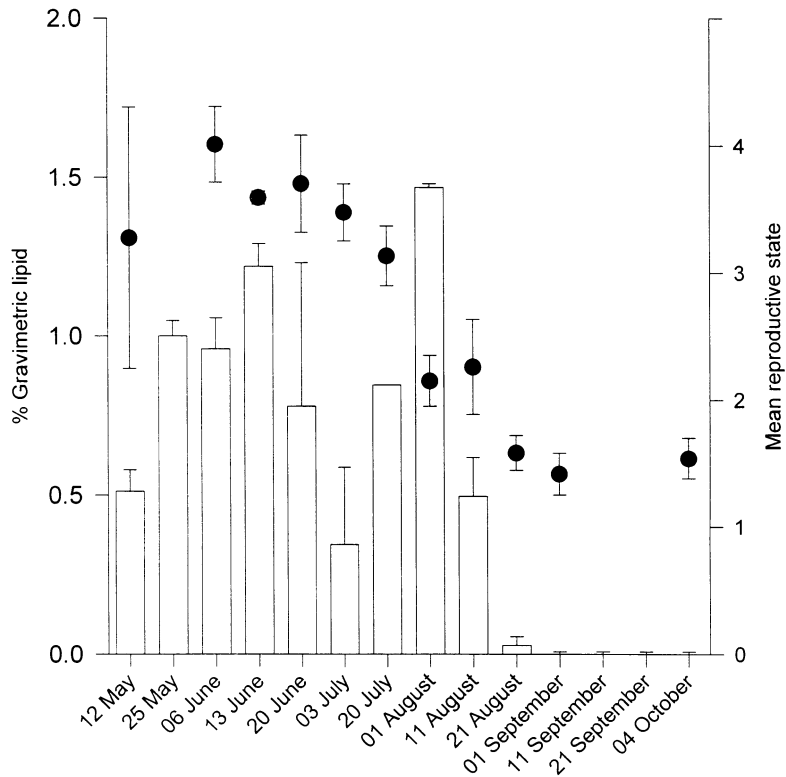


Fig. 2. Mean percent (\pm S.E.M.) lipid content (solid circles) of zebra mussels and mean (\pm S.E.M.) reproductive state (open bars) of female zebra mussels where: 1 = immature, sexes indistinguishable; 2 = immature, sexes separate; 3 = germinal vesicle in less than 50% of oocytes; 4 = germinal vesicle in more than 50% of oocytes; 0 = spent.

Table 3

Mean (\pm S.E.M.) % of total wet mass of neutral lipid (NLI), polar lipid (PLI) and total lipids (TLI) as quantified by Iatroscan

Date	Mussel reproductive status	% NLI	% PLI	% TLI
12 May	Immature	0.48 \pm 0.02	0.75 \pm 0.02	1.23 \pm 0.02
20 June	Gravid	0.48 \pm 0.03	0.87 \pm 0.03	1.35 \pm 0.06
3 July	Spent	0.67 \pm 0.12	0.81 \pm 0.05	1.48 \pm 0.17
20 July	Immature	0.57 \pm 0.03	0.82 \pm 0.03	1.39 \pm 0.08
1 August	Gravid	0.32 \pm 0.02	0.77 \pm 0.06	1.10 \pm 0.07
21 August	Spent	0.20 \pm 0.01	0.67 \pm 0.03	0.87 \pm 0.04

Concentrations of all but the two lowest K_{ow} compounds surveyed (HCB and PCB 31/28) were correlated with neutral lipid content ($r^2 = 0.64$; $P < 0.05$). By contrast, total gravimetric lipid and total Iatroscan lipid correlated poorly with all chemicals except PCB 118 ($r^2 = 0.60$; $P < 0.05$). In addition, contaminant concentrations normalized to neutral lipid content gradually increased from May through to August for all but the lowest and highest K_{ow} compounds (Fig. 3), though no such patterns were evident for values adjusted for gravimetric lipid content (Table 2).

Mussels were induced to spawn in the laboratory on 20 June, 20 July and 11 August. In all instances, no significant differences in gravimetric lipid content or chemical concentrations between spent male and female mussels were detected

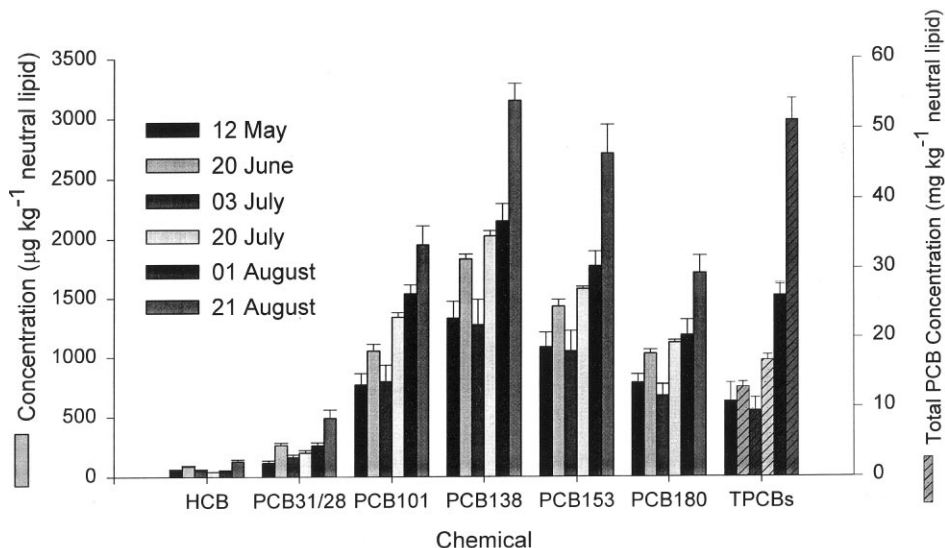


Fig. 3. Temporal variation in mean (\pm S.E.M.) contaminant concentrations normalized to neutral lipid content of each of two low-, mid- and high- K_{ow} compounds and total PCBs. The six dates represent sexually immature, gravid and spent mussels for the two spawning cycles.

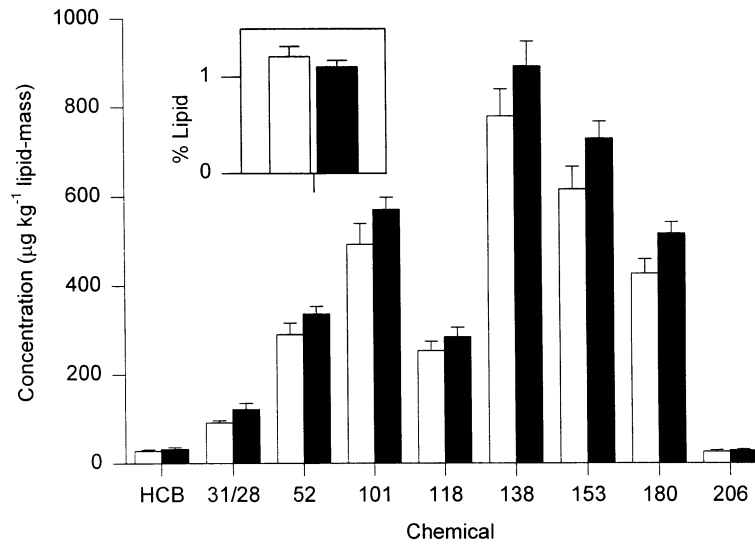


Fig. 4. Differences in mean (+ S.E.M.) contaminant body burdens between gravid (open bars) and spent (solid bars) mussels for animals spawned in the laboratory on 20 June, 20 July and 11 August. Differences in percentage lipid are shown in the inset.

(paired t -tests, $\alpha = 0.005$). As such, spent male and female concentrations were averaged for each replicate. Gravid and spent mussels did not differ with respect to either gravimetric lipid content or concentrations (gravimetric lipid-adjusted) of any of the nine chemicals (Fig. 4; paired t -tests; $\alpha = 0.005$).

4. Discussion

The zebra mussel population surveyed in this study was skewed toward females (61.1–65.6%), in contrast to findings of Garton and Haag (1993) who reported an equal sex ratio for a Lake Erie population. Other European and North American populations also exhibit a slight bias (55–60%) toward females (Walz, 1978; Mackie, 1991). The mussel population surveyed here exhibited two spawning events during 1995. Multiple spawning events in *D. polymorpha* have been documented for other North American and European populations (Borcherding, 1991; Mackie, 1993; Gist et al., 1997).

Gravimetrically-determined total lipid levels reported herein are comparable to published values (1.2–1.8% wet-weight; van der Oost et al., 1988; Brieger and Hunter, 1993). Moreover, temporal variation in total lipid content exhibited by zebra mussels in this study (Fig. 2) is consistent with previous observations from Lakes Erie and St. Clair (Dermott et al., 1993; Nalepa et al., 1993). Variability in lipid content is thought to reflect changes in food quality and quantity (Walz, 1979; Garton and Haag, 1993). Workers have also attributed changes in lipid content in

bivalves to reproductive activity, though without direct confirmation (Pieters et al., 1980; Zandee et al., 1980; Nalepa et al., 1993). In this study, the association between reproductive status and lipid content does not appear to be as direct as traditionally assumed. For example, on August 1st lipid content was low even though mussels were gravid and nearly fully mature (Fig. 2). Furthermore, there was minimal loss of lipid observed in lab-spawned *D. polymorpha* (Fig. 4). This finding was surprising considering that zebra mussels may lose up to 50% of dry mass during gamete release (Borcherding, 1991; Sprung, 1991; Garton and Haag, 1993). Although oocytes from fish and other mussels generally contain a higher lipid content than somatic tissue (Pieters et al., 1980; Miller, 1993), estimates for zebra mussel oocytes vary from 9.8 to 78.8% of dry mass (Sprung, 1989). That lipid content did not significantly decrease for laboratory-spawned mussels in this study suggests that oocytes contained relatively low amounts of lipid. Lipid levels, for mussels with low-lipid oocytes, could be related more to other physiological or environmental factors than to reproductive activity. Separation of lipid content and reproductive status on different principal components analysis (PC analysis) lends additional support to this concept.

It is also interesting that limnological factors such as chlorophyll *a* and lake temperature were separated from reproduction by PC analysis. While temperature is thought to be a proximate cue for spawning, variation in phytoplankton abundance is believed to have a more direct effect (Ram and Nichols, 1993; Gist et al., 1997). Variation in chlorophyll concentration was unrelated to mussel reproduction (Table 1; Fig. 2), though it is possible that chlorophyll did not provide an accurate measure of food available to mussels.

Concentrations of organochlorine contaminants in zebra mussels reported here are three to five times higher than those for similar-sized mussels found elsewhere in the basin. In this study, maximum concentrations ranged from 100 to 3000 $\mu\text{g kg}^{-1}$ lipid for HCB and PCB 138, respectively, (Table 2), whereas, concentrations of the same chemicals were less than 200 $\mu\text{g kg}^{-1}$ lipid elsewhere in the basin (Morrison et al., 1995; Mazak et al., 1997). The relatively high concentrations observed here almost certainly reflect the close proximity of the sampling site to the Detroit River, the primary source of contaminants to Lake Erie (Kauss and Hamdy, 1985; Oliver and Bourbonniere, 1985).

Results from this study demonstrate that contaminant concentrations in zebra mussels are significantly influenced by physiological and environmental factors. Variation in mid- and high- K_{ow} compounds were related to reproductive state and to precipitation, whereas, that of the least hydrophobic compound surveyed (HCB) was related to lipid content and reproductive cycle only. However, direct links are difficult to make. The increase in contaminant concentrations on 20 July occurred prior to sexual maturation of the mussels or of any decrease in lipid content, both of which occurred on 1 August (Table 2; Fig. 2). The increase in chemical concentration did, however, follow two consecutive days of the heaviest rainfall measured over the study period. That other heavy showers did not result in an increase in contaminant body burdens indicates that a precipitation threshold may exist below which contaminant influx to rivers and the lake is negligible.

Chemical concentrations in mussels may have increased initially owing to storm activity and remained high, thereafter, due to a rapid decrease in lipid. Time to essential steady state for organochlorine contaminants in zebra mussels ranges from 20 to 85 days (Morrison et al., 1995). Between 20 July and 01 August, total lipid content declined ca. 36% (Fig. 2). A second, smaller increase in concentration (14–29%) on 21 August was matched by a further 28% reduction in total lipid content (Table 2, Fig. 2). Neutral lipid content fell 70% between 3 July and 21 August (Table 3). The decline in lipid but not chemical concentrations may result in non-equilibrium conditions in which the fugacity of the organism exceeds that of its environment. This condition would particularly affect dynamics of high- K_{ow} compounds in mussels with low lipid levels because of slow elimination rates (Bruner et al., 1994; Morrison et al., 1995). These findings suggest that mussels may achieve only quasi-steady-state conditions due to fluctuations in lipid levels and reproductive state (Pizza and O'Connor, 1983; Capuzzo et al., 1989).

Temporal variation in PCB concentrations in this study corresponded more with neutral lipid content than with either gravimetric total lipid or Iatroscan total lipid. These patterns suggest that equilibrium partitioning may depend more on neutral than on total lipids, and that variation in neutral lipids may partially explain anomalies in lipid-adjusted contaminant concentrations. For example, Hummel et al. (1989) detected no difference in PCB concentrations on a neutral lipid (fat) basis in tissues of *Mytilus edulis*. Likewise, Delbeke et al. (1995) found that 50% of the variability in PCB concentrations observed in an aquatic food web could be explained by normalization to neutral lipid content. The increase in neutral lipid-adjusted chemical concentrations during our study (Fig. 3) suggests that neutral lipids decreased faster than chemicals could be eliminated from mussels.

Reproductive (i.e. gonad) development influenced body burdens of moderately high- K_{ow} PCBs, though it was decoupled from actual spawning events (Fig. 2; Table 2). For example, no significant differences were observed in concentrations of the nine selected compounds among gravid and spent individuals (Fig. 4). Hummel et al. (1989) observed that mantle tissues accounted for up to 40% of PCB body burden in *M. edulis* and speculated that a substantial proportion of contaminants may be shed during spawning. Spawning-induced elimination of organochlorine contaminants has been reported in female fishes (Westin et al., 1983; Miller, 1994). The effect of spawning by males on contaminant dynamics is less certain. Spawning by paddlefish (*Polyodon spathula*) resulted in greater losses in PCB concentration in females than in males because the mass of milt was less than that of roe (Gundersen and Pearson, 1992). However, spawning resulted in similar losses of tetrachlorobiphenyl by male and female rainbow trout (*Oncorhynchus mykiss*; Guiney et al., 1979). We did not detect differences between male and female mussels with regard to percentage lipid content and contaminant concentration before and after spawning. However, this result must be interpreted with caution, considering that spawning in females may not have been complete.

In summary, limnological factors (including chlorophyll *a* concentration) did not explain temporal variation in contaminant concentrations. Rather, contaminant levels corresponded best with variation in mussel reproductive development, spawning cycle, precipitation and neutral lipid level in mussels.

Acknowledgements

We thank Dr Rodica Lazar and David Qiu (Great Lakes Institute, University of Windsor) for their assistance in performing contaminant and lipid analysis and Joann Cavaletto (Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan) for performing the Iatroskan analysis. Andrew Bially and Robert Coulas assisted with field work. Drs Doug Haffner and Mike Weis provided insightful comments on this project. This study was supported financially by a grant from the Natural Sciences and Engineering Research Council of Canada to H.J.M. and by an Ontario Ministry of Environment and Energy (666G) grant to H.J. MacIsaac, G.D. Haffner and J.J. Ciborowski.

References

- Borcherding, J., 1991. The annual reproductive cycle of the freshwater mussel *Dreissena polymorpha* Pallas in lakes. *Oecologia* 87, 208–218.
- Brieger, G., Hunter, R.D., 1993. Uptake and depuration of PCB 77, PCB 169 and hexachlorobenzene by zebra mussels (*Dreissena polymorpha*). *Ecotoxicol. Environ. Saf.* 26, 153–165.
- Bruner, K.A., Fisher, S.W., Landrum, P.F., 1994. 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. *J. Great Lakes Res.* 20, 725–734.
- Capuzzo McDowell, J., Farrington, J.W., Rantamaki, P., Clifford, C.H., Lancaster, B.A., Leavitt, D.F., 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.
- Claissie, D., 1989. Chemical contamination of French coasts: the results of 10 years mussel watch. *Mar. Pollut. Bull.* 20, 523–528.
- Connell, D.W., 1988. Bioaccumulation behavior of persistent organic chemicals with aquatic organisms. *Rev. Environ. Contam. Toxicol.* 101, 117–154.
- Delbeke, K., Teklemariam, T., de la Cruz, E., Sorgeloos, P., 1995. Reducing variability in pollution data: the use of lipid classes for normalization of pollution data in marine biota. *Int. J. Environ. Anal. Chem.* 58, 147–162.
- Dermott, R., Mitchell, J., Murray, I., Fear, E., 1993. Biomass and production of zebra mussels (*Dreissena polymorpha*) in shallow waters of northeastern Lake Erie. In: Nalepa, T.F., Schloesser, D.W. (Eds.), *Zebra Mussels: Biology, Impacts and Control*. Lewis Publishers, Boca Raton, pp. 399–413.
- Duursma, E.K., Nieuwenhuize, J., van Liere, J.M., 1984. Organochlorine contamination of the Dutch delta region as 'watched' by mussels. *Water Sci. Technol.* 16, 619–622.
- Fallon, M.E., Horvath, F.J., 1985. Preliminary assessment of contaminants in soft sediments of the Detroit River. *J. Great Lakes Res.* 11, 373–378.
- Fisher, S.W., Gossiaux, D.C., Bruner, K.A., Landrum, P.F., 1993. Investigations of the toxicokinetics of hydrophobic contaminants in the zebra mussel (*Dreissena polymorpha*). In: Nalepa, T.F., Schloesser, D.W. (Eds.), *Zebra Mussels: Biology, Impacts and Control*. Lewis Publishers, Boca Raton, pp. 465–490.
- Furlong, E.T., Carter, D.S., Hites, R.A., 1988. Organic contaminants in sediments from the Trenton Channel of the Detroit River, Michigan. *J. Great Lakes Res.* 14, 489–501.
- Garton, D.W., Haag, W.R., 1993. Seasonal reproductive cycles and settlement patterns of *Dreissena polymorpha* in western Lake Erie. In: Nalepa, T.F., Schloesser, D.W. (Eds.), *Zebra Mussels: Biology, Impacts and Control*. Lewis Publishers, Boca Raton, pp. 111–128.
- Giese, F., Krüger, A., 1992. Biomonitoring of organochlorines in surface waters of Berlin and the Lake Stechlin. In: Neumann, D., Jenner, H.A. (Eds.), *The Zebra Mussel, Dreissena polymorpha*. Gustav Fischer Verlag Publishers, New York, pp. 245–254.

- Gist, D.H., Miller, M.C., Brence, W.A., 1997. Annual reproductive cycle of the zebra mussel in the Ohio River: a comparison with Lake Erie. *Arch. Hydrobiol.* 138, 365–379.
- Goldberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker, P.L., Risebrough, R.W., Robertson, W., Schneider, E., Gamble, E., 1978. The mussel watch. *Environ. Conserv.* 5, 101–125.
- Guiney, P.D., Melancon, M.J. Jr., Lech, J.J., Peterson, R.E., 1979. Effects of egg and sperm maturation and spawning on the distribution and elimination of a polychlorinated biphenyl in rainbow trout (*Salmo gairdneri*). *Toxicol. Appl. Pharmacol.* 47, 261–272.
- Gundersen, D.T., Pearson, W.D., 1992. Partitioning of PCBs in the muscle and reproductive tissues the paddlefish, *Polyodon spathula*, at the falls of the Ohio River. *Bull. Environ. Contam. Toxicol.* 49, 455–462.
- Hummel, H., UitOudeGroeneveld, J.P., Nieuwenhuize, J., van Liere, J.M., Bogaards, R.H., de Wolf, L., 1989. Relationship between PCB in mussels *Mytilus edulis*. *Mar. Environ. Res.* 28, 438–493.
- Hummel, H., Bogaards, R.H., Nieuwenhuize, J., de Wolf, L., van Liere, J.M., 1990. Spatial and seasonal differences in the PCB content of the mussel *Mytilus edulis*. *Sci. Total Environ.* 92, 155–163.
- Hurlbert, S.H., White, M.D., 1993. Experiments with freshwater invertebrate zooplanktivores: quality of statistical analyses. *Bull. Mar. Sci.* 53, 128–153.
- Kates, M., 1986. Techniques in lipidology: isolation, analysis and identification of lipids. In: Burdon, R.H., van Knippenberg, P.H. (Eds.), *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. III, Pt. 2. Elsevier, Amsterdam, p. 464.
- Kauss, P.B., Hamdy, Y.S., 1985. Biological monitoring of organochlorine contaminants in the St. Clair and Detroit Rivers using the introduced clam, *Elliptio complanatus*. *J. Great Lakes Res.* 11, 247–263.
- Kraak, M.H.S., Scholten, M.C.T., Peeters, W.H.M., de Kock, W.C., 1991. Biomonitoring of heavy metals in the western European Rivers Rhine and Meuse using the freshwater mussel *Dreissena polymorpha*. *Environ. Pollut.* 74, 101–114.
- Lazar, R., Edwards, R.C., Metcalfe, C.D., Metcalfe, T., Gobas, F.A.P.C., Haffner, G.D., 1992. A simple, novel method for the quantitative analysis of coplanar (non-orthosubstituted) polychlorinated biphenyls in environmental samples. *Chemosphere* 25, 493–504.
- Leach, J.H., 1993. Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs in western Lake Erie. In: Nalepa, T.F., Schloesser, D.W. (Eds.), *Zebra Mussels: Biology, Impacts and Control*. Lewis Publishers, Boca Raton, pp. 381–397.
- Lind, O.T., 1979. Handbook of Common Methods in Limnology. C.V. Mosby, St. Louis, pp. 199.
- Mackay, D., 1982. Correlation of bioconcentration factors. *Environ. Sci. Technol.* 16, 274–278.
- Mackie, G.L., 1991. Biology of the exotic zebra mussel, *Dreissena polymorpha* in relation to native bivalves and its potential impact in Lake St. Clair. *Hydrobiologia* 219, 251–268.
- Mackie, G.L., 1993. Biology of the zebra mussel (*Dreissena polymorpha*) and observations of mussel colonization on unionid bivalves in Lake St. Clair of the Great Lakes. In: Nalepa, T.F., Schloesser, D.W. (Eds.), *Zebra Mussels: Biology, Impacts and Control*. Lewis Publishers, Boca Raton, pp. 153–165.
- Mazak, E.J., MacIsaac, H.J., Servos, M.R., Hesslein, R., 1997. Influence of feeding habits on organochlorine contaminant accumulation in waterfowl on the Great Lakes. *Ecol. Applic.* 7, 1133–1143.
- Mersch, J., Jeanjean, A., Spor, H., Pihan, J.-C., 1992. The freshwater mussel *Dreissena polymorpha* as a bioindicator for trace metals, organochlorines and radionuclides. In: Neumann, D., Jenner, H.A. (Eds.), *The Zebra Mussel, Dreissena polymorpha*. Gustav Fischer Verlag, New York, pp. 245–254.
- Miller, M.A., 1993. Maternal transfer of organochlorine compounds in Salmonines to their eggs. *Can. J. Fish. Aquat. Sci.* 50, 1405–1413.
- Miller, M.A., 1994. Organochlorine concentration dynamics in Lake Michigan Chinook salmon (*Oncorhynchus tshawytscha*). *Arch. Environ. Contam. Toxicol.* 27, 367–374.
- Morrison, H., Yankovich, T., Lazar, R., Haffner, G.D., 1995. Elimination rate constants of 36 PCBs in zebra mussels (*Dreissena polymorpha*) and exposure dynamics in the Lake St. Clair–Lake Erie corridor. *Can. J. Fish. Aquat. Sci.* 52, 2574–2582.
- Nalepa, T.F., Cavaletto, J.F., Ford, M., Gordon, W.M., Wimmer, M., 1993. Seasonal and annual variation in weight and biochemical content of the zebra mussel (*Dreissena polymorpha*) in Lake St. Clair. *J. Great Lakes Res.* 19, 541–552.

- Nichols, S.J., 1993. Spawning of zebra mussels (*Dreissena polymorpha*) and rearing of veligers under laboratory conditions. In: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra Mussels: Biology, Impacts and Control. Lewis Publishers, Boca Raton, pp. 315–329.
- Oliver, B.G., Bourbonniere, R.A., 1985. Chlorinated contaminants in surficial sediments of Lakes Huron, St. Clair and Erie: implications regarding sources along the St. Clair and Detroit Rivers. J. Great Lakes Res. 11, 366–372.
- Parrish, C.C., 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. 44, 722–731.
- Phillips, D.J.H., 1980. Quantitative Aquatic Biological Indicators. Their Use to Monitor Trace Metal and Organochlorine Pollution. Applied Science, London, p. 448.
- Pieters, H., Kluytmans, J.H., Zandee, D.I., Cadée, G.C., 1980. Tissue composition and reproduction of *Mytilus edulis* in relation to food availability. Neth. J. Sea Res. 14, 349–361.
- Pizza, J.C., O'Connor, J.M., 1983. PCB dynamics in Hudson River striped bass. II. Accumulation from dietary sources. Aquat. Toxicol. 3, 313–327.
- Ram, J.L., Nichols, S.J., 1993. Chemical regulation of spawning in the zebra mussel (*Dreissena polymorpha*). In: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra Mussels: Biology, Impacts and Control. Lewis Publishers, Boca Raton, pp. 307–314.
- Ram, J.L., Crawford, G.W., Walker, J.U., Mojares, J.J., Patel, N., Fong, P.P., Kyozuka, K., 1993. Spawning in the zebra mussel (*Dreissena polymorpha*): activation by internal or external application of serotonin. J. Exp. Zool. 265, 587–598.
- Secor, C.L., Mills, E.L., Harshbarger, J., Kuntz, H.T., Gutenmann, W.H., Lisk, D.J., 1993. Bioaccumulation of toxicants, element and nutrient composition and soft tissue histology of zebra mussels (*Dreissena polymorpha*) from New York State waters. Chemosphere 26, 1559–1575.
- Sokal, R.R., Rohlf, F.J., 1995. Biometry. W.H. Freeman, San Francisco, CA, p. 887.
- Sprung, M., 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: abundance, growth, mortality and food demands. Arch. Hydrobiol. 115, 537–561.
- Sprung, M., 1991. Costs of reproduction: a study on metabolic requirements of the gonads and fecundity of the bivalve *Dreissena polymorpha*. Malacologia 33, 63–70.
- Sprung, M., 1995. Physiological energetics of the zebra mussel *Dreissena polymorpha* in lakes. I. Growth and reproductive effort. Hydrobiologia 304, 117–132.
- Tabachnick, G.G., Fidell, L.S., 1996. Using Multivariate Statistics. Harper-Collins College Publishers, New York, p. 880.
- Tanabe, S., Tatsukawa, R., Phillips, D.J.H., 1987. Mussels as bioindicators of PCB isomers and congeners in green-lipped mussels (*Perna viridis*) in Hong Kong waters. Environ. Pollut. 47, 41–62.
- van der Oost, R., Heida, H., Opperhuizen, A., 1988. Polychlorinated biphenyl congeners in sediments, plankton, molluscs, crustaceans and eel in a freshwater lake: implications of using reference chemicals and indicator organisms in bioaccumulation studies. Arch. Environ. Contam. Toxicol. 17, 721–729.
- Walz, N., 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. II. Reproduction. Arch. Hydrobiol. 55 (Suppl.), 106–119.
- Walz, N., 1979. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. V. Seasonal and nutritional changes in the biochemical composition. Hydrobiologia 55 (Suppl.), 235–254.
- Westin, D.T., Olney, C.E., Rogers, B.A., 1983. Effects of parental and dietary PCBs on survival, growth, and body burdens of larval striped bass. Bull. Environ. Contam. Toxicol. 30, 50–57.
- Wilkinson, L., Hill, M., Vang, E., 1992. SYSTAT: Statistics, version 5.2 edition. Evanston, IL: SYSTAT, p. 724.
- Zandee, D.I., Kluytmans, J.H., Zurburg, W., Pieters, H., 1980. Seasonal variations in biochemical composition of *Mytilus edulis* with reference to energy metabolism and gametogenesis. Neth. J. Sea Res. 14, 1–29.