BIOPROCESSING OF ALGAE AND TRANSFER OF
HYDROPHOBIC CONTAMINANTS BY ZEBRA MUSSELS

(DREISSENA POLYMORPHA)

A Thesis

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by

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To My Parents and My Husband
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CHAPTER I
INTRODUCTION

The zebra mussel (*Dreissena polymorpha*), a bivalve mollusc, originates in Russia near the Caspian Sea. Since its introduction into Lake St. Clair in the 1980s by accidental ballast discharge (Hebert et al. 1989), the zebra mussel has spread throughout the Great Lakes and other major waterways in America, including the St. Lawrence Seaway and the Hudson, Illinois, Mississippi, Ohio, Arkansas and Tennessee Rivers (Sea Grant 1994). The population density of zebra mussels has been reported as high as 400,000 individuals per square meter in the western basin of Lake Erie in 1990 (MacIsaac et al. 1991). The rapid expansion of the zebra mussel results from its free swimming veliger larva, strong byssus attachment, and its high reproductive rate (Turner 1990).

Because of the large population and high filtering rate (about one liter of water per zebra mussel per day), the zebra mussel has been recognized as a good candidate for accumulating hydrophobic contaminants from water column. Also, the high filtering rate allows zebra mussels to filter more particles than their
nutritional requirement, some of the filtered particles are expelled as pseudofeces. Furthermore, unassimilated contaminants in pseudofeces and feces could serve as a source of contaminants to benthic organisms, which consume feces and pseudofeces, thus increasing the risk of contaminant exposure to benthic organisms living in or near mussel colonies.

The influence of the zebra mussel on aquatic ecosystems has caused great concerns for environmental scientists. Evidence has shown that zebra mussels have changed the community structure, nutrient cycling, and pelagic-benthic biomass balance in Lakes Erie and St. Clair (Beeton 1995). It is also possible that zebra mussels could affect contaminate cycling (Bruner et al. 1994; Fisher et al. 1993). Because of these concerns, it is imperative to determine the role of zebra mussels in contaminant transfer in the aquatic environment.

The accumulation of hydrophobic pollutants by aquatic organisms including zebra mussels depends on a number of physical and biological factors. Among these, lipid content is regarded as a pivotal factor in regulating the accumulation of hydrophobic contaminants (Geyer et al. 1985). The accumulation of polychlorinated biphenyls (PCBs) by aquatic organisms in the St. Lawrence Estuary was significantly correlated with lipid content of organisms (Gagnon et al. 1990). Similarly, in San Francisco Bay, it was demonstrated that 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT) and its metabolites were more greatly
accumulated in *Dwarf perch* with a 6.41% lipid content than in *Staghorn Sculpin*, which had a low lipid content (1.87%) (Earnest and Benville 1971).

Zebra mussels have an average lipid content of about 11.4% (Walz 1979) which facilitates the accumulation of hydrophobic contaminants. The uptake and elimination of hydrophobic contaminants have been compared between the zebra mussel and *Mytilus edulis* (Fisher *et al.* 1993), whose lipid content is less than 2% (Renberg *et al.* 1986). The bioconcentration of hydrophobic chemicals by zebra mussels was significantly higher than that of *Mytilus edulis*. The difference in lipid content was most likely responsible for the difference in bioconcentration because the hydrophobic contaminants would be ultimately deposited in the lipid. Thus, the lipid-rich characteristic of the zebra mussel makes them more likely to accumulate hydrophobic contaminants.

For zebra mussels and other filter-feeding bivalves, there are two major contaminant exposure routes. One is accumulation of freely dissolved chemicals from water during filtration. The second is ingestion of contaminated materials such as algae and suspended sediments. Uptake of freely dissolved contaminants from water is a passive process, and compounds can quickly diffuse into the body of zebra mussels by crossing the gill membrane followed by deposition into the tissue (Fisher *et al.* 1993; Bruner *et al.* 1994). In contrast, uptake of contaminants from suspended particles is relatively slow because the chemicals associated with ingested materials must desorb from the particles and pass
through the gut epithelium before deposition into the tissue (Gobas et al. 1993). But, this route may be more biologically significant because of the higher contaminant concentration of particles compared with water.

No agreement has been reached as to which exposure route dominates the contaminant uptake by mussels. The relative importance of the two routes depends on many factors such as the concentration of contaminants in the water or adsorbed to the particles, the chemical properties of the contaminants, and the digestibility of the particles. Since hydrophobic contaminants have a strong tendency to adsorb to organic-rich matter such as algae, the contaminant accumulation by means of ingestion could be a more important exposure route than uptake of contaminants from water. For some aquatic organisms such as fish, the uptake of contaminants from food instead of freely dissolved contaminants in water was suggested to be the major pathway for hydrophobic contaminants accumulation (Connolly and Pedersen 1988; Bruggeman et al. 1984). Thomann and Connolly (1984) reported that for a top predator such as the Lake Michigan trout, 99% of the polychlorinated biphenyl (PCB) in the body was accumulated by consumption of contaminated food rather than by uptake from water. Similarly, the removal of hydrophobic chemicals by zebra mussels via ingestion of particles could be a major source of contaminant exposure when the chemical concentration in water is low (Bruner et al. 1994).
After accumulation by zebra mussels, contaminants can be channeled into food chains via two separate routes: (1) by direct trophic transfer, in which contaminated zebra mussels are consumed by predators at higher trophic levels. Thus, the assimilated contaminants in the zebra mussel tissue will be transferred to the predators, or (2) by indirect trophic transfer, where pseudofeces and feces having unassimilated contaminants will be biodeposited onto the sediments. Subsequent ingestion of the contaminated fecal and pseudofecal materials by detritivores will introduce toxicants to the food chain. Both routes may ultimately lead to significant contaminant exposure to organisms at higher trophic levels.

Bioprocessing of Algae

For the zebra mussels, bioprocessing of suspended particles such as algae means the filtration of algae followed by ingestion, assimilation, or ejection of some particles as pseudofeces and feces. Filtered algae have three distinctive fates when passing through mussels. First, filtered algae can be ingested and assimilated into tissue to provide zebra mussels with nutrition. Second, some portion of the filtered algae may be ingested but not assimilated, and expelled as feces. Third, some portion of the filtered algae may be wrapped in mucous and ejected through the inhalant siphon as pseudofeces prior to ingestion. The pseudofeces are the aggregation of algal particles, and their larger size makes
them less likely to resuspend than single cells (Berg et al. 1993). As a result, they readily deposit onto the sediments. The amount of pseudofeces and feces produced by active filtering mussels can be very large. In Uchinskoye water reservoir, the *D. polymorpha* population can produce 48.8 tons (dry weight) feces and pseudofeces per day (Izvekova and Lvova-Katchanova 1972). The huge amount of pseudofeces and feces can increase the amount of food to benthic communities, resulting in greater numbers of benthic organisms. For example, the population of macroinvertebrates such as *Gammarus fasciatus* has increased greatly in zebra mussel colonies (Dermott and Barton 1991; Griffiths 1993).

Bioprocessing is complicated by variation in algal species and algal cell concentrations (Berg et al. 1993). These elements influence the filtering rate, the total algal mass filtered, and the relative production of pseudofeces and feces. For example, the filtering rate of zebra mussels decreased as the algal concentration increased for six species of algae (*Chlamydomonas, Pedinomonas, Casmarium, Euglena, Pleodorina, and Pediastrum*) (Morton 1971). In addition, algal concentration can change the relative production of pseudofeces. A positive linear relationship was found between the amount of pseudofeces produced and suspended matter for zebra mussels (Reeders and Vaate 1992).

The filtering rate and pseudofeces production determine the amount of algae ingested and ingestion efficiency. For *Mytilus edulis* L., *Cerastoderma Edule* (L)
and *Venerupis pullastra* (Montagu), two strategies have been used to cope with high algal concentrations: (1) reducing filtering rate, and (2) increasing production of pseudofeces (Foster-Smith 1975). Both strategies help these organisms to control ingestion and maintain a constant amount of filtered food to supply their nutritional requirement. Understanding the mechanisms by which zebra mussels control their ingestion is important to understand how zebra mussels process contaminants sorbed to particles. For instance, if no pseudofeces are produced at high contaminated food concentrations, contaminants would be effectively accumulated by zebra mussels. However, if pseudofeces are produced at high algal concentrations, contaminants could be passed to pseudofeces, which may be consumed by benthic invertebrates. Thus, the ultimate fate of particle-associated contaminants in the food chain may depend on the types of particles filtered.

**Assimilation and Transfer of Hydrophobic Contaminants**

Polychlorinated biphenyls (PCBs) and 1,1-dichloro-2,2-bis[4-chlorophenyl]ethylene (DDE) are hydrophobic organic compounds (HOCs). Their high lipophilicity facilitates bioaccumulation by organisms (Swackhammer and Skoglund 1993).

Zebra mussels can accumulate hydrophobic contaminants by ingesting contaminated algae. The extent of contaminant accumulation via ingestion is
determined by contaminant assimilation efficiency (AE), gut retention, and feeding route (Harkey et al. 1994). Contaminant AE is the fraction of the ingested contaminants retained by the organisms. It is defined as the ratio of the amount of compounds assimilated through the gut to the total amount of chemicals ingested. Contaminant AE is a critical parameter for estimating the bioavailability of contaminants to organisms (Luoma et al. 1992). Efforts have been made to improve the accuracy of environmental models by measuring contaminant AE (Thomann et al. 1992).

Contaminant bioaccumulation and contaminant AE are affected by physicochemical characteristics of chemicals, including chemical persistence in the environment, biological affinity, water solubility, toxicity to organisms and compound lipophility (Matsumura 1985). For hydrophobic contaminants, the most important characteristic related to accumulation is compound lipophility, which can be represented by the octanol/water partition coefficient ($K_{ow}$). $K_{ow}$ has been widely used as an effective indicator for the tendency of chemicals to partition into the lipid of organisms (Thomann 1989). $K_{ow}$ is also used in contaminant bioaccumulation models to predict the amount of contaminants bioaccumulated by organisms (Thomann 1989). Generally, compounds with higher log $K_{ow}$ values will have strong tendency to be accumulated by aquatic organisms (Mackay 1982). In contrast, a negative correlation exists between compound partition coefficients and contaminant AE for some highly
hydrophobic compounds in fish and molluscs (Means et al. 1980; Jahan-Parwar 1990). For the zebra mussels, contaminant %AE for DDT, BaP and TCBP increased in the order of 44.9%, 53.0% and 77.6%, while the $K_{ow}$ values increased in the order of TCBP, BaP and DDT (Bruner et al. 1994). In an assessment of bioaccumulation and fecal elimination of hydrophobic organic chemicals by fish, an empirical formula between the $K_{ow}$ and contaminant AE was derived:

$$\frac{1}{AE} = 5.3 \times 10^{-8} K_{ow} + 2.3$$ (Gobas et al. 1988)

The equation suggests that the contaminant AE are constant for chemicals with log $K_{ow}$ values smaller than 7, but decrease for compounds with log $K_{ow}$ greater than 7. Thus, compound $K_{ow}$ is an important characteristic for determining the assimilation of hydrophobic contaminants via ingestion.

Contaminant AE may also be influenced by different food types. Because of the relative digestibility of different food types, gut passage time (GPT) will vary for different food. GPT is considered to be an effective indicator for estimating contaminant assimilation efficiency (Kofod et al. 1989). The shorter the GPT for food and contaminants absorbed to food, the fewer the contaminants will diffuse from food, and the smaller the contaminant AE. For the same food, the amount of ingested food would also affect the GPT and contaminant AE. For instance, guppies contaminant AE decreased when an identical amount of
chemicals was fed but with a large food volume. The reduction in contaminant AE resulted from a more rapid egestion rate (Clark and Mackay 1991).

Hypotheses and Objectives

The zebra mussels play an important role in contaminant cycling primarily through bioprocessing of suspended particles such as algae, which determines contaminant transfer by zebra mussels. Therefore, bioprocessing of algae and transfer of contaminants via ingestion of contaminated algae were examined. Bioprocessing of algae, contaminant AE and transfer of contaminants to pseudofeces and feces are discussed in Chapter 2 and Chapter 3 respectively.

Hypotheses

Chapter 2: Differences in the structure and morphology of algae will result in different filtering rate, total amount of algae filtered, and the algal assimilation efficiencies. In addition, the filtering rate and the pseudofeces production will vary with different algal concentrations for each species of algae.

Chapter 3: Contaminant assimilation efficiencies will vary when zebra mussels ingest different algae spiked with different chemicals. The different contaminant assimilation efficiencies would lead to the different contaminant concentrations in pseudofeces and feces. Also, different algal concentrations (same chemical concentration) will result in different contaminant assimilation efficiencies.
Objectives

Chapter 2: The bioprocessing experiments will estimate the effect of algal concentrations and algal structure and morphology on the filtering rate, the total amount of algae filtered, the pseudofeces production and algal assimilation efficiency of the zebra mussels using three species of algae (Chlorella vulgaris, Chlamydomonas reinhardtii and Ankistrodesmus falcatus). The mechanisms that zebra mussels control ingestion will also be investigated.

Chapter 3: Mussel contaminant assimilation efficiencies of four chemicals (2,2',4,4'-tetrachlorobiphenyl (TCBP), 3,3',4,4',5-pentachlorobiphenyl (PCBP), 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP) and 1,1-dichloro-2,2-bis[4-chlorophenyl]ethylene (DDE)) will be determined at two different algal concentrations of Chlorella vulgaris and Chlamydomonas reinhardtii respectively based on chemical mass-balance model. In addition, the transfer of contaminants to the feces and pseudofeces is assessed by measuring the contaminant concentrations in feces and pseudofeces.
CHAPTER II

BIOPROCESSING OF ALGAE BY ZEBRA MUSSEL, *DREISSENA POLYMORPHA*: EFFECT OF ALGAL CONCENTRATION AND ALGAL SPECIES ON THE BIOPROCESSING

INTRODUCTION

Since its introduction in the 1980s, the zebra mussel, *Dreissena Polymorpha*, has become a dominant exotic species in the North American Great Lakes (Hebert *et al.* 1989). Like other nonindigenous species, zebra mussel is significantly altering the preexisting ecological relationships and environmental processes in the aquatic ecosystems (Sea Grant 1995). For example, proliferation of zebra mussels has led to a shift in algal community structure in Saginaw Bay, Lake Huron, which may further affect the benthic food webs in that system (Pillsbury and Lowe 1995). In addition, zebra mussel filtration activity may interfere with pelagic communities by changing the concentration of dissolved oxygen, nitrogen and phosphorus, and by sedimentation of algae through the formation of pseudofeces and feces (Culver *et al.* 1991).
Zebra mussels have also been reported to influence contaminant cycling (Fisher et al. 1993; Bruner et al. 1994). Filtration of contaminated particles and water is thought to be a critical mechanism for zebra mussels to accumulate chemicals (Yankcovich and Haffner 1992). For example, contaminants such as polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) were assimilated into mussel tissue from the ingested particles, while unassimilated contaminants were passed to pseudofeces and feces (Bruner et al. 1994; Reeder and Vaate 1992), which provided a potential contaminant exposure to other benthic organisms.

The adverse effect of the zebra mussel on ecosystems is closely related to its ability to filter large amounts of water and suspended particles. Bivalves, including zebra mussels, can remove suspended particles from water and return some portions of them to water in the forms of pseudofeces and feces (Bayne and Newell 1983). Therefore, to understand zebra mussels’ effect on the nutrient and contaminant cycling in the natural environment, both of the amount of food materials filtered from the water and the amount of the food lost through rejection of pseudofeces and feces must be assessed (Widdows et al. 1979).

For the zebra mussels, bioprocessing of suspended particles such as algae involves the filtration of algae followed by ingestion, assimilation, or expulsion of some particles as pseudofeces and feces. Bioprocessing is complicated and affected by many environmental factors such as temperature, oxygen content, salinity, and food size and quality (Bayne et al. 1976; Sprung and Rose 1988).
Previous studies showed that zebra mussels and other suspension-feeding bivalves varied their filtering rate and pseudofeces production in response to the changes in food concentrations or food species (Berg et al. 1993; Reeders and Vaate 1992). In a study of the effect of suspension concentration on the filtration rate and pseudofeces production, *Mytilus Edulis* L., *Cerastoderma Edule* (L.) and *Venerupis Pullastra* (Montagu) demonstrated two strategies to cope with high particle concentrations: 1) decreasing filtering rate to limit the amount of algae filtered, and 2) increasing pseudofeces production, which would expel any extra filtered particles (Foster-Smith 1975). Other species such as *Mercenaria mercenaria* and *Crassostrea virginica* use same mechanisms to control ingestion at high particle concentrations to maintain a constant ingestion rate (Bricelj and Malouf 1984; Haven and Morales-Alamo 1966).

The mechanisms for bivalves to regulate ingestion would affect the contaminant cycling. If organisms decrease their filtering rate without producing pseudofeces at high contaminated particle concentrations, most of the contaminants would be accumulated into the tissue, and fewer would be expelled in the feces. However, if some pseudofeces are produced at high contaminated particle concentration, some contaminants would be directly sedimented in the pseudofeces (Reeders and Vaate 1992). The contaminants expelled with pseudofeces and feces will be available for ingestion and assimilation to benthic organisms.
Zebra mussels filter suspended particles indiscriminately, then filtered food particles are selected based on algal characteristics such as particle size and shape (Bayne and Newell 1983). Thus, bioprocessing should likely vary with algal species having different algal structure and morphology. Because of the importance of zebra mussels in the nutrient and contaminant cycling, the bioprocessing of algae was examined. The filtering rate and the pseudofeces production at varying algal concentrations were evaluated to examine the mechanisms of bioprocessing. Three species of algae (*Ankistrodesmus falcatus*, *Chlamydomonas reinhardii* and *Chlorella vulgaris*) differing in size, motility, shape and composition were used to determine the effect of algal species on zebra mussel bioprocessing.

**MATERIALS AND METHODS**

**Materials**

**Organisms**

Zebra mussels (*Dreissena polymorpha*) were collected by SCUBA divers from littoral zones in North Bay, Kelly's Island, Lake Erie. Collections varied from early spring to early fall. The mussels were kept cool and moist during transport to Ohio State University, Columbus, Ohio. Mussels were held in 200 L aquaria filled with aerated carbon-filtered tap water at 12°C. A suspension of mixed diatoms (Oyster Diet B, Coast Seafood Company) or live algal mixture
*Chlorella vulgaris, Chlamydomonas reinhardii* and *Ankistrodesmus falcatus* was fed to zebra mussels every two days (about 3.3 g food/ per 1000 zebra mussels). Water quality in aquaria was monitored daily for temperature, pH, and dissolved oxygen. Sixty percent of the water in the aquaria was changed weekly. Discarded water was treated with chlorine bleach (50 ppm) for 24 h to prevent the dissemination of veligers into local waterways. At least two weeks before the experiments, zebra mussels were transferred to incubators for acclimation to test conditions (temperature, pH and alkalinity [130mg/L as CaCO₃ and hardness, 170mg/L as CaCO₃]).

**Algae**

Three species of algae (*Chlorella vulgaris, Chlamydomonas reinhardii* and *Ankistrodesmus falcatus*) were used. The initial populations of each algal species were obtained from Carolina Biological Supply. Monocultures of each algal species were maintained in 9:1 Bold's Basal Medium under a light regime of 16:8 h (light: dark) (Nichols and Bold 1965). The purity of the monoculture was checked weekly using the light-microscope.

**Methods of Bioprocessing Experiments**

For 48-72 h prior to the experiment, 20±1 cm-long acclimated zebra mussels were allowed to reattach to glass petri dishes placed in 4-L aquaria filled with Hard Standard Reference Water (HSRW) at 22°C (USEPA 1975). Zebra mussels were starved for 48 h before the experiments so that they would expel all materials from their guts.
On the day of the experiment, acclimated zebra mussels were transferred to a 4-L aquarium. Only healthy zebra mussels that reattached to the petri dishes in the previous 48 h, gaped their shells, and extended their inhalant and exhalent siphons quickly were used in the experiments.

The algal cell concentrations in algal culture were determined with a Coulter Counter Model ZBI (Hialeah, Florida), then volumes of algal culture used in the experiments were determined according to the algal cell concentrations in the algal culture and experimental algal solution. An algal solution was prepared by adding algal culture to 13 L Hard Standard Reference Water (HSRW) (pH 8.4-8.5). After mixing for 30 minutes, a uniform solution was obtained. The solution was divided into three replicate groups. Triplicate water samples from each group were taken to measure the algal mass density. Algal mass density was determined by filtering 50-200ml of solution through preweighed 0.5 μm glass filters (Fisher Scientific, Pittsburgh, PA), then dried in the oven for at least 24 h at 60°C.

In each of three replicate groups, 500 mL algal solutions were added to each of eight 1000 mL beakers. Then one zebra mussel was placed into each beaker. Beakers were held in a darkened incubator for 3 h at 22°C. After 3 h, zebra mussels were removed from the exposure beakers and placed into other beakers filled with clean HSRW. Pseudofeces and feces produced during the 3 h exposure were collected separately in each group. In addition, in each group, 50-200 mL water samples taken from each beaker were mixed together in a 4-L
aquarium and triplicate 100-300 mL subsamples were filtered through preweighed 0.5 μm glass filters to measure the algal mass density as described above.

During the next 72 h, feces and pseudofeces were collected separately. For each group, the pseudofeces and feces from each set of 8 beakers were pooled to determine the total pseudofeces and feces production. Pseudofeces are green and wrapped in mucous in the shape of balls. In contrast, feces are black-green and pellet-shaped. All the pseudofeces and feces produced in the 3 h exposure and 72 h purge time were removed by a pipette, filtered through the preweighed 0.5 μm glass filters, then dried in the oven for at least 24 h at 60°C to determine the dry weight.

Algal mass densities determined at the beginning ($T_0$) and the end ($T_3$) of the exposure time were used to calculate the total algal mass filtered by the zebra mussels and the filtering rate. Also, the total pseudofeces and feces produced during the 3 h exposure and 72 h purging period were used to calculate algal assimilation efficiency.

If we make the following assumptions: (1) the reduction of algal concentration in the water over time is due to zebra mussel filtration, (2) the algal cells remain suspended in the water because of the turbulence produced by zebra mussel filtration, and (3) the filtering rate is constant for zebra mussels (Berg et al. 1993), then the total algae filtered by a zebra mussel (B, μg) and the filtering rate (FR, mL min⁻¹) can be calculated using the following equations:
\[ B = (C_0 - C_t) \times V \]

\[ FR = [(\ln C_0 - \ln C_t) \times V]/T \]

where \( C_0, C_t \) = the average of algal concentration (ug mL\(^{-1}\), dry weight) in each beaker at the beginning and end of the exposure period respectively.

\( V \) = the volume (500 mL) of the algal solution exposed to a zebra mussel.

\( T \) = the exposure time (180 min.).

Algal assimilation efficiency is defined as the ratio of the algal mass assimilated to the total algal mass ingested in the gut by the organisms. The algal percent assimilation efficiency is calculated as:

\[ \%AE = \frac{(B-P-F)/(B-P)}{X100} \]

\( F \) = the total dry weight of feces (ug ind\(^{-1}\)). \( P \) = the total dry weight of pseudofeces (ug ind\(^{-1}\)).

The assumption of the model is that the filtered algal particles have three distinctive fates as they pass through the zebra mussels: (1) filtered particles can be ingested and assimilated; (2) some portions of the filtered algae may be ingested but not assimilated, and expelled as feces; and (3) some filtered algae can be wrapped in mucous and ejected through the inhalant siphon as pseudofeces.

Data Analysis

Regression analysis was used to test the correlation of algal concentration with bioprocessing parameters such as filtering rate, the production of pseudofeces and the total algae filtered. Single-Factor ANOVA (analysis of
variance) F-test was employed to analyze the significant difference of some parameters such as algal assimilation efficiency at different algal concentrations. Also, Tukey's multiple comparison was applied to investigate the difference between individual variables. The Tukey's method utilizes the studentized range distribution (HSD). An alpha level of 0.05 was used in the data analysis as the level of significance.

RESULTS

_Zebra Mussel Bioprocessing of Chlamydomonas reinhardtii_

_Zebra mussels used in the experiments were collected in late May of 1994. The total algal mass filtered by the zebra mussels, the filtering rate, the total amount of pseudofeces, and the algal percent assimilation efficiency are summarized in Table 1. The total algae filtered, the filtering rate, and the amount of pseudofeces varied with algal concentration (Figs. 1-3).

Within the algal concentration range of 2.19 μg mL⁻¹ to 8.09 μg mL⁻¹, the total mass of algae filtered by zebra mussels (Table 1 and Figure 1) showed no significant difference (ANOVA, P>0.05) except at 2.19 μg mL⁻¹, the lowest algal concentration in the experiment. This implies that the mass of algae filtered by the zebra mussels increased to a maximum and then held steady.

The filtering rate (FR) was based on the removal of algae from water by zebra mussels. Although high individual variances masked the effect of algal concentration on mussel filtering rate, the data suggested a decreasing trend in
filtering rate with increasing algal concentration (Fig. 2, FR = 3.45e⁻⁰.¹³C, \( r^2 = 0.70, n = 8 \), C: algal concentration).

Unlike the negative relationship between the filtering rate and algal concentration, the amount of pseudofeces (PW) was positively correlated with the algal concentration within the tested algal concentration range (Figs 3).

The algal assimilation efficiency was calculated based on the algal mass-balance model discussed above. The percent algal assimilation efficiency (%AE) ranged from 82.5 to 89.6% and did not vary significantly with different algal concentrations (ANOVA, P > 0.05) (Table 1).

**Zebra Mussel Bioprocessing of Chlorella vulgaris**

Zebra mussels used in the experiment were collected in late May of 1995. Within the algal concentration range of 1.99-8.63 μg mL⁻¹, the filtering rate varied from 2.22 mL min⁻¹ to 1.02 mL min⁻¹ (Table 2). The filtering rate was negatively correlated with algal concentration (Fig. 4, FR = 2.47e⁻⁰.¹¹C, \( r^2 = 0.60, n = 8 \), C: algal concentration).

Unlike the relationship between the algal concentration and the total algae filtered by the zebra mussels fed Chlamydomonas reinhardtii, the amount of filtered Chlorella vulgaris (B) increased with the increasing algal concentration (Fig. 5). The correlation of the total algae filtered with algal concentration was:

\[ B = 359.3(±138.9) + 119.6(±25.7)C \ (r^2 = 0.78, n = 8, C: \text{algal concentration}) \]
The amount of pseudofeces (PW, ug ind.\(^{-1}\)) produced by mussels was strongly correlated with algal concentration (PW=82.6(±16.1)C-83.5(±86.9), \(r^2=0.82, n=8, C:\) algal concentration) (Fig. 6).

Algal assimilation efficiencies ranged from 86.0 to 89.9\%, and were independent of algal concentration over the experiment range (ANOVA, \(P>0.05\)) (Table 2).

**Zebra Mussel Bioprocessing of *Ankistrodesmus falcatus***

Zebra mussels used in the experiments were collected in late May of 1994. Unlike the zebra mussels fed *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, the filtering rate of zebra mussels fed *Ankistrodesmus falcatus* was not negatively related to algal concentrations, but exhibited a maximum at a cell concentration of 5.6 \(\mu\)g mL\(^{-1}\) (Fig. 7). The single-factor ANOVA one-way F test indicated that the filtering rate significantly varied with the algal concentration (\(F=8.23, P=0.000 <0.05\))(Table 3).

The trend between the total *Ankistrodesmus falcatus* filtered and algal concentration was different from that of zebra mussels fed *Chlamydomonas reinhardtii*, but resembled that of zebra mussels fed *Chlorella vulgaris*. That is, the total algae (B) filtered increased with the increasing algal concentrations (Fig. 8, \(B=176.2(±40.4)C+77.3(±236.5), r^2=0.76, n=8, C:\) algal concentration).

In comparison with the pseudofeces produced by zebra mussels fed *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, zebra mussels produced a greater percentage of pseudofeces when fed *Ankistrodesmus falcatus* at
corresponding algal concentrations. Further, zebra mussels fed *Ankistrodesmus falcatus* produced pseudofeces more quickly than those when fed *Chlamydomonas reinhardtii* or *Chlorella vulgaris*. However, the correlation of the amount of pseudofeces (PW) with the algal concentration was similar to that of zebra mussels fed the other two species of algae, and positively correlated with the algal concentration (Figs.9) \( \text{PW} = 103.1(\pm25.3)C + 22.4(\pm148.3), r^2 = 0.74, n=7 \). Data collected at the concentration of 7.42 µg mL\(^{-1}\) was excluded from the analysis because of its large standardized residual in the data set.

Because of the large amount of pseudofeces produced by zebra mussels fed *Ankistrodesmus falcatus*, only a small amount of algae was ingested, and as a result, fewer feces were produced. The minimal amount of feces relative to the greater amount of pseudofeces made it difficult to collect feces. In some experiments, no feces were found in the 72 hour purging period. Consequently, the algal assimilation efficiencies of *Ankistrodesmus falcatus* calculated by the algal mass-balance model were essentially 100% (Table 3).

**DISCUSSION**

The hypothesis that the three species of algae (*Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Ankistrodesmus falcatus*) would produce different responses in zebra mussel bioprocessing, and the algal concentration would affect the filtering rate and the pseudofeces production are supported by the results.
Table 1. The total algae filtered (B) (ug ind.^-1), the filtering rate (FR) (mL min.^-1 ind.^-1), the total pseudofeces (PW) (ug ind.^-1), and percent algal assimilation efficiency (%AE) at different algal concentrations (C_a) (ug mL.^-1, DW) for zebra mussels fed *Chlamydomonas reinhardtii*. The numbers in parenthesis are standard errors. Within a column, numbers with common superscripts are not significantly different (P > 0.05).

<table>
<thead>
<tr>
<th>C_a (ug mL.^-1, DW)</th>
<th>B (ug ind.^-1)</th>
<th>FR (mL min.^-1 ind.^-1)</th>
<th>PW (ug ind.^-1)</th>
<th>%AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.19</td>
<td>555.8^a (63.0)</td>
<td>2.01^d (0.31)</td>
<td>51.4^a (5.6)</td>
<td>87.5^a (2.5)</td>
</tr>
<tr>
<td>4.36</td>
<td>1072.6^b (34.4)</td>
<td>1.88^bcd (0.09)</td>
<td>192.0^b (20.2)</td>
<td>89.0^a (1.0)</td>
</tr>
<tr>
<td>4.50</td>
<td>1353.6^b (33.6)</td>
<td>2.57^d (0.11)</td>
<td>303.9^bc (20.4)</td>
<td>88.0^a (1.1)</td>
</tr>
<tr>
<td>5.04</td>
<td>1260.0^b (99.3)</td>
<td>1.95^bcd (0.23)</td>
<td>280.9^bc (32.5)</td>
<td>82.5^a (0.5)</td>
</tr>
<tr>
<td>5.78</td>
<td>1381.6^b (16.7)</td>
<td>1.87^abcd (0.03)</td>
<td>368.6^c (49.9)</td>
<td>87.2^a (1.1)</td>
</tr>
<tr>
<td>7.09</td>
<td>1219.1^b (130.5)</td>
<td>1.18^ab (0.15)</td>
<td>258.5^bc (39.0)</td>
<td>89.2^a (1.1)</td>
</tr>
<tr>
<td>7.87</td>
<td>1381.8^b (84.3)</td>
<td>1.21^abc (0.09)</td>
<td>326.2^bc (26.9)</td>
<td>89.6^a (1.8)</td>
</tr>
<tr>
<td>8.09</td>
<td>1209.0^b (114)</td>
<td>1.03^a (0.20)</td>
<td>377.8^c (8.5)</td>
<td>85.7^a (2.4)</td>
</tr>
</tbody>
</table>
Figure 1. The relationship between algal concentration and total algae filtered by zebra mussels during exposure time. Vertical bars represent standard errors. (Alage: *C. reinhardtii*).

Figure 2. The relationship between algal concentration and zebra mussel filtering rate. Vertical bars represent standard errors. (Algae: *C. reinhardtii*).
Figure 3. The relationship between algal concentration and amount of pseudofeces produced by zebra mussels. Vertical bars represent standard errors. (Algae: *C. reinhardtii*)
Table 2. The total algae filtered (B) (ug ind.\(^{-1}\)), the filtering rate (FR) (mL min.\(^{-1}\) ind.\(^{-1}\)), the total pseudofeces (PW) (ug ind.\(^{-1}\)), and percent algal assimilation efficiency (%AE) at different algal concentrations (C\(_a\)) (ug mL\(^{-1}\), DW) for zebra mussels fed *Chlorella vulgaris*. The numbers in parenthesis are standard errors. Within a column, numbers with common superscripts are not significantly different (P>0.05).

<table>
<thead>
<tr>
<th>C(_a) (ug mL(^{-1}), DW)</th>
<th>B (ug ind.(^{-1}))</th>
<th>FR (mL min(^{-1})ind.(^{-1}))</th>
<th>PW (ug.ind(^{-1}))</th>
<th>%AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.99</td>
<td>547.1(^a) (11.6)</td>
<td>2.22(^a) (0.04)</td>
<td>107.3(^a) (5.2)</td>
<td>88.0(^a) (1.1)</td>
</tr>
<tr>
<td>3.75</td>
<td>836.2(^bc) (31.7)</td>
<td>1.65(^bcd) (0.10)</td>
<td>230.0(^b) (6.8)</td>
<td>86.0(^b) (0.7)</td>
</tr>
<tr>
<td>3.89</td>
<td>984.6(^cde) (43.2)</td>
<td>1.97(^ab) (0.10)</td>
<td>263.8(^bcd) (24.0)</td>
<td>88.1(^a) (1.6)</td>
</tr>
<tr>
<td>4.04</td>
<td>688.5(^ab) (28.5)</td>
<td>1.16(^def) (0.05)</td>
<td>241.5(^abc) (8.8)</td>
<td>88.7(^a) (1.5)</td>
</tr>
<tr>
<td>5.45</td>
<td>1043.9(^de) (8.6)</td>
<td>1.34(^def) (0.04)</td>
<td>357.0(^bcd) (18.9)</td>
<td>87.0(^a) (1.6)</td>
</tr>
<tr>
<td>6.10</td>
<td>937.2(^cd) (79.8)</td>
<td>1.02(^e) (0.10)</td>
<td>239.6(^b) (18.1)</td>
<td>88.6(^a) (0.9)</td>
</tr>
<tr>
<td>6.52</td>
<td>1332.1(^f) (46.5)</td>
<td>1.47(^cde) (0.11)</td>
<td>554.8(^e) (30.3)</td>
<td>89.9(^a) (1.0)</td>
</tr>
<tr>
<td>8.63</td>
<td>1329.3(^f) (114.0)</td>
<td>1.02(^ef) (0.11)</td>
<td>673.3(^e) (82.6)</td>
<td>86.9(^a) (1.6)</td>
</tr>
</tbody>
</table>
Figure 4. The relationship between algal concentration and zebra mussel filtering rate. Vertical bars represent standard errors. (Algae: *C. vulgaris*)
Figure 6. The relationship between algal concentration and amount of pseudofeces produced by zebra mussels. Vertical bars represent standard errors. (Algae: *C. vulgaris*)
Table 3. The total algae filtered (B) (µg ind⁻¹), the filtering rate (FR) (µL min⁻¹ ind⁻¹), the total pseudofeces (PW) (µg ind⁻¹), and the algal percent assimilation efficiency (%AE) at different algal concentrations (Cₐ) (µg mL⁻¹, DW) for zebra mussels fed Ankistrodesmus falcatus. The numbers in parenthesis are standard errors. Within a column, numbers with common superscripts are not significantly different (P>0.05).

<table>
<thead>
<tr>
<th>Cₐ (µg mL⁻¹, DW)</th>
<th>B (µg ind⁻¹)</th>
<th>FR (µL min⁻¹ ind⁻¹)</th>
<th>PW (µg ind⁻¹)</th>
<th>%AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.62</td>
<td>286.0a (30.0)</td>
<td>1.22abcd (0.16)</td>
<td>118.2a (12.0)</td>
<td>90.67</td>
</tr>
<tr>
<td>3.23</td>
<td>579.5ab (22.4)</td>
<td>1.24abde (0.06)</td>
<td>308.0ab (32.3)</td>
<td>94.66</td>
</tr>
<tr>
<td>4.65</td>
<td>724.2bc (9.9)</td>
<td>1.04ab (0.02)</td>
<td>434.4abc (20.4)</td>
<td>100*</td>
</tr>
<tr>
<td>5.08</td>
<td>1077.1ed (96.6)</td>
<td>1.55abcde (0.19)</td>
<td>611.0bcd (60.6)</td>
<td>89.75</td>
</tr>
<tr>
<td>5.57</td>
<td>1448.5def (85.8)</td>
<td>2.05f (0.18)</td>
<td>925.1d (73.4)</td>
<td>100*</td>
</tr>
<tr>
<td>7.42</td>
<td>1640.6f (62.3)</td>
<td>1.63bcdef (0.09)</td>
<td>690.3ed (35.7)</td>
<td>100*</td>
</tr>
<tr>
<td>7.73</td>
<td>1159.6de (106.2)</td>
<td>1.00a (0.11)</td>
<td>717.5ed (81.4)</td>
<td>96.73</td>
</tr>
<tr>
<td>8.28</td>
<td>1349.0def (116.0)</td>
<td>1.10abc (0.12)</td>
<td>866.2d (123.9)</td>
<td>100*</td>
</tr>
</tbody>
</table>

Number with * is estimated based on the algal mass-balance. No statistical analysis is performed in the algal assimilation efficiencies.
Figure 7. The relationship between algal concentration and zebra mussel filtering rate. Vertical bars represent standard error. (Algae: *A.falcatus*)

Figure 8. The relationship between algal concentration and total algae filtered by zebra mussels during exposure time. Vertical bars represent standard errors. (Algae: *A.falcatus*)
Figure 9. The relationship between algal concentration and amount of pseudofeces produced by zebra mussels. Vertical bars represent standard errors. (Algae: *A. falcatus*)
Filtering rate and pseudofeces production are the two important parameters describing the bioprocessing of suspended particles by zebra mussels. They determine the amount of suspended particles removed from water by zebra mussels, and also determine the biomass transferred to benthic organisms associated with zebra mussels.

The filtering rate has been reported in a wide variety of bivalves. Two methods are usually involved in measuring filtering rate, a direct method and an indirect method. The direct method separates the exhalant water flow from surrounding water by inserting a tube into the exhalant siphon of the organisms, and then measuring the flow rate. This approach is used for larger bivalves such as deposit-feeding clams. The indirect method measures the removal rate of suspended particles from water, and is the most appropriate method when working with small filter-feeding bivalves.

Within the algal concentrations tested, the mussel filtering rate ranged from 1.00 mL min\(^{-1}\) to 2.57 mL min\(^{-1}\) for the three species of algae studied. The results are similar to previous measurement of zebra mussels' filtering rate using *Chlamydomonas reinhardtii* and *Pandorian morum* (Berg et al. 1993), but are much lower than those of Chen's (1993), who used *Chlamydomonas reinhardtii* to measure filtering rate (FR is about 4 mL min\(^{-1}\) for 60 min. exposure). This may be due to the different exposure time in the two experiments. The filtering rate is known to be affected by the duration that mussels are exposed to algae (Chen 1993). In addition, the filtering rate
among the three species of algae was not significantly different, suggesting that
the filtering rate is independent of the three algal species. Filtering rate simply
measures the removal rate of algae from water, it does not consider the food
selection by the mussels after filtering particles, thus differences among different
algal species might not be expected.

Although limited data are available on the effect of algal species on filtering
rate, extensive accounts have focused on the relationship between the particle
concentration and filtration rate of bivalves. It is agreed that bivalves show
different filtering patterns in response to the change of particle concentration
(Morton 1983; Jørgensen 1990). For a large number of bivalves such as
*Crassostrea virginica*, *Crassostrea gigas* and *Mytilus edulis*, the filtering rate
generally decreased when the particle concentration increased (Loosanoff and
Engle 1947; Widdows *et al.* 1979). However, for some species the filtering rate
did not change with the particle concentration (Berg *et al.* 1993). Winter (1977)
gave a more complete summary of the relationship between food concentration
and filtering rate. Bivalves tend to change filtering rates following three phases:
(1) an increase in filtering rate in response to the initial exposure to low threshold
of particle concentration; (2) a plateau phase over some optimal particle level;
and (3) a decrease in filtering rate following a further increase of particle
concentration, also coinciding with increased pseudofeces production.
Therefore, the range of food concentration must be specified to properly interpret
the relationship between filtering rate and particle concentration.
For each algal species, zebra mussels had different responses when fed high concentrations of algae. Although large variations obscured the effect of algal concentration on the filtering rate, a trend of decreasing filtering rate following the increasing algal concentration was observed for zebra mussels fed *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. In contrast, the filtering rate appeared to closely follow the description of Winter (1977) when zebra mussels were exposed to * Ankistrodesmus falcatus*, and exhibited an apparent maximum near the mean algal concentration used.

The mechanisms that cause zebra mussels to reduce the filtering rate following an increase in algal concentration are not clearly understood, but several biological or physiological factors may be responsible. For zebra mussels and other bivalves, food collection is by filtration (Morton 1983). Therefore, zebra mussels must continuously filter to catch food from the water. It is reasonable that at low algal concentrations, zebra mussels should increase the filtering rate to obtain the “diluted food”. As reported by Schulte (1975), low algal concentration may stimulate the filtering activity of some bivalves, whereas, at the high algal concentrations, the filtering mechanism was disturbed by the overload of suspended particles to the gills, and would lead to the decreased filtering rate. Mathers (1974) suggested for the Oyster *O. edulis* and *C. angulata*, a high algal concentration may provoke secretion of mucous and block the ctenidia that were responsible for the food collection.
In considering the effect of algal concentration on the filtering rate, it is also important to identify the change in the pumping activity of zebra mussels (Sprung and Rose 1988). Although the pumping rate and filtering rate have the same dimension (volume per time), they have different meanings. Filtering rate is the rate that particles are cleared from water by organisms, whereas, pumping rate means the water volume pumped through the feeding apparatus of organisms per unit time (Winter 1978). The two parameters are equal when the particle retention efficiency by the gill is 100%. Pumping is generally not continuous, and is more frequently interrupted at high particle concentrations than at low particle concentrations. Also, the temporary restriction of the diameter of the exhalant siphon at high algal concentration could directly decrease the pumping rate (Foster-Smith 1976). Because the food particles are delivered to the feeding apparatus with water, it is very reasonable to assume that the depressed pumping activity will result in a decreased filtering rate.

Unlike that of zebra mussels fed *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, the filtering rate of zebra mussels fed *Ankistrodesmus falcatus* was not negatively dependent on algal concentrations used in the experiment. The filtering rates increased at initial increasing algal concentrations, after reaching a maximum, they decreased with the increased algal concentrations. The filtering rate curve vs. algal concentrations is similar to the description of Winter (1977). At low algal concentration, the little increase of algal concentrations would stimulate the filtration activity, so the filtering rate increases. When the algal
concentration reaches a certain point, the increased algal concentration would disturb the filtration activity as discussed above, and the filtering rate decreases.

Pseudofeces were produced when zebra mussels were fed *Chlamydomonas reinhardtii*, *Chlorella vulgaris* and *Ankistrodesmus falcatus*. The pseudofeces are the particles that are filtered but not ingested by the zebra mussels and other bivalves (Ten Winkled and Davids 1983; Walz 1978). These filtered particles are expelled from the mantle cavity via the inhalant siphon. Possible explanations for the formation of pseudofeces are: (1) zebra mussels remove extra particles filtered to keep the gills working (Foster-Smith, 1975), (2) pseudofeces are related to particle selection. That is, zebra mussels would discard inorganic particles or particles hard to digest (Küppers and Mühlenberg 1981).

The dependence of pseudofeces production on particle concentration is well documented. For example, a relationship between the amount of pseudofeces (PSF) and the suspended matter (dry matter content, DMC, mg L\(^{-1}\)) was reported for 15mm-long zebra mussels:

\[
PSF = -0.28 + 0.48 \times DMC
\]

\((R^2 \sim 0.99, n=5, \text{range: } 4-87 \text{ mg.L}^{-1} \text{ DMC})\) (Reeders and Vaate 1992)

Our results confirm the results of these studies. Despite the difference of the amount of pseudofeces produced by zebra mussels fed three species of algae, the amount of pseudofeces were always positively related to the algal concentration.

At the high algal concentration, the filtering rate decreased tracking the increasing pseudofeces production when mussels ingested the three algal species.
The increased pseudofeces with the increased algal concentration could result from the zebra mussels' regulation of ingestion. For the zebra mussels and other bivalves, food collection is by filtration of suspended particles from the water column (Morton 1983). However, the food obtained by the zebra mussels is not unlimited. "Satiation" has been reported for some bivalves, such as Copepods (Corner et al. 1973). Satiation is the level at which the bivalves maintain a maximum steady rate of ingestion. Once satiation occurs, zebra mussels could not digest more algae. However, zebra mussels still continue to filter algae from water. Therefore, extra algae filtered would be inhaled and ejected via inhalant siphons as pseudofeces. At high algal concentrations, more algae are filtered than that at low algal concentration. Consequently, more pseudofeces are produced. It is also possible that the higher the algal concentration, the greater amount of the algae overloading the pallial feeding apparatus. Consequently, a larger percentage of filtered algae would be rejected as pseudofeces (Foster-Smith 1975).

Zebra mussels fed Ankistrodesmus falcatus produced more pseudofeces than the zebra mussels fed Chlamydomonas reinhardtii and Chlorella vulgaris. This implies that zebra mussels preferred Chlamydomonas reinhardtii and Chlorella vulgaris to Ankistrodesmus falcatus. Many species of bivalves are believed to have the ability to select and preferentially ingest some particles and discard other particles (Ward et al. 1994). In fact, bivalves have evolved the ciliation to collect, transfer, sort and select food (Morton 1983). In this study,
the results implied that mussel’ preference on food may be based on the algal digestibility, size, motility and other characteristics such as algal components. *Chlamydomonas reinhardtii* is considered to be a good food for suspension feeders such as zebra mussels because of its suitable spherical shape, size, and motility, which keep them moving in the water. In addition, their cell wall contains no cellulose which makes them easier to digest (Brendelberger and Jürgens 1993). *Chlorella vulgaris* has a similar size and shape as *Chlamydomonas reinhardtii*, but it is nonmotile, and also it has cellulose in its cell wall. Therefore, *Chlorella vulgaris* is thought to be less digestible than *Chlamydomonas reinhardtii*. However, the current and previous studies demonstrated that *Chlorella vulgaris* could be digested by zebra mussels. In contrast, *Ankistrodesmus falcatus* has distinctive characteristics that differentiate it from the other two algal species. Its individual cells are long spindled shape. Its length is about 10-20 times than that of the other two algal species. Also, its cells are sometimes aggregated in small groups. Therefore, the large size and volume of *Ankistrodesmus falcatus* may make them difficult to digest and most of them will be discarded as pseudofeces. This is supported by the large amount of pseudofeces produced by mussels when fed *Ankistrodesmus falcatus*.

The trend of increased pseudofeces in response to the extremely high algal concentration is limited. In a study of feeding activity of *Mytilus edulis*, Widdows *et al.* (1979) reported that the production of pseudofeces increased with algal concentration, but the pseudofeces production decreased after
reaching very high algal concentrations. The reason is that the extremely high algal concentration inhibits the normal filtration activity. As a result, the total algae filtered by the mussels decrease, and the amount of pseudofeces declines as well. In a preliminary experiment, zebra mussels were exposed to a very high concentration of *Chlamydomonas reinhardtii* (11.05 μg mL⁻¹), almost twice the average of the algal concentrations used in the filtering rate experiments. In this case, the filtering rate was significantly lower than the filtering rate reported in our study. The lower filtering rate was accompanied by the decrease of total algae filtered and pseudofeces production. Extremely high algal concentrations were not tested in this experiment, but it could be concluded that much higher algal concentrations could overload the gill and disturb filtration.

In sum, zebra mussels use both reduction in filtering rate and increase in pseudofeces production to control algal ingestion at high algal concentrations. However, the strategies are algal-species-dependent. For example, the zebra mussel simultaneously decreased the filtering rate and increased the pseudofeces production at high algal concentrations when fed *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. For the mussels fed *Ankistrodesmus falcatus*, they increased the pseudofeces production independent of the change of filtering rates.

Two patterns were observed for the total algal filtered by mussels following the increase in algal concentration. For the zebra mussels fed *Chlamydomonas reinhardtii*, the total algae filtered at different algal concentrations approached a plateau, indicating satiation of ingestion. At low algal concentrations, the
amount of algae filtered may not satisfy the food requirement. Thus, the zebra mussels must filter continuously to obtain more algae. When algae concentrations reach a certain threshold, the algae filtered would reach the satiation point for the mussels. Consequently, they would decrease filtering rate to limit the influx of algae.

For the zebra mussels fed *Ankistrodesmus falcatus*, the total algae filtered by the zebra mussels increased as the algal concentration increased. One possible explanation may be that the zebra mussels produced much more pseudofeces when filtering *Ankistrodesmus falcatus*, and also the pseudofeces were produced more quickly compared with the mussels fed the other two species of algae. So, the amount of algae filtered was always below the satiation requirement of food. Therefore, zebra mussels must filter more algae to compensate for the loss of algae as pseudofeces.

The reason that the total algae filtered increased with the increasing concentration of *Chlorella vulgaris* could not be explained clearly. Because *Chlorella vulgaris* is reported to be digested by the mussels, it should have shown the same pattern as that of mussels fed *Chlamydomonas reinhardtii*. It may be that zebra mussels fed *Chlorella vulgaris* require more time to reach the satiation point. That is, if the mussels were allowed to filter *Chlorella vulgaris* for more than 3 h, the total algae filtered by the zebra mussels at different algal concentrations would have the same pattern as that of mussels fed *Chlamydomonas reinhardtii*. 
If we compare the total algae (B) filtered by the zebra mussels fed three different algal species, they were not significantly different from each other at corresponding concentrations. Like the filtering rate, the total algae (B) filtered by the mussels are simple estimates of the amount of algae removed from the water, and do not account for the amount of pseudofeces produced. So B do not meaningfully describe the assimilation ability of mussels. Consequently, B should be considered with assimilation efficiency simultaneously so that organism assimilation ability could be estimated comprehensively.

Food assimilation efficiencies have been widely reported for bivalves. However, conflicting results have been reported for the relationship between food concentration and assimilation efficiency. For example, the assimilation efficiency of phytoplankton by *Putamocorbula amurensis* was inversely related to algal concentration (Werner and Hollibargh 1993). In contrast, Berg et al. (1993) reported that the algal assimilation efficiency by the zebra mussels did not change with the algal concentration. While other authors suggested that assimilation efficiency is related to the amount of algae filtered, not the algal concentration. For example, by accounting of $^{32}$P in algae, feces and pseudofeces, Foster-Smith (1975) showed that algal assimilation efficiency is inversely related to the total algae ingested rather than related to the algal concentrations. Actually, it is very difficult to obtain meaningful assimilation efficiency in the bivalves. This may be due to the difficulty in getting reliable data
of the exact amount of food ingested and completely recovering the pseudofeces and feces (Morton 1983).

In light of the algal mass-balance model, the algal assimilation efficiency of zebra mussel fed *Chlamydomonas reinhardtii* and *Chlorella vulgaris* were very similar, and did not vary with the algal concentrations. Bayne and Newell (1983) regarded that assimilation efficiency of around 80% was typical for mussels fed pure algal suspensions. So the algal assimilation efficiencies of *Chlamydomonas reinhardtii* and *Chlorella vulgaris* measured in the study were reasonable. In contrast, for the zebra mussels fed *Ankistrodesmus falcatus*, the algal assimilation efficiencies were higher than that of zebra mussel fed the other two species of algae. As discussed in the results section, this may be due to the difficulty in collecting the limited amount of feces. Also, it could be attributed to the fact that mussels must increase the assimilation efficiencies to compensate the small amount of algae ingested in the gut.

In sum, zebra mussels can greatly filter suspended particles such as algae from water system, and also can expel some filtered algae as feces and pseudofeces. The filtration activity of zebra mussels has significant ecological effect due to the trophic interaction (Asmus and Asmus 1991). Because of enormous amount of phytoplankton and zooplankton filtered by zebra mussels, zebra mussels may compete food with some fish, which may be responsible for the decreased population of yellow perch in Lake Erie (Sea Grant 1996). In addition, zebra mussel filtration may influence contaminant cycling in fresh water
system (Geyer et al. 1982; Fisher et al. 1993; Bruner et al. 1994). Zebra mussels not only accumulate contaminants into their tissue, but also pass unassimilated contaminants to pseudofeces and feces. Therefore, factors affecting zebra mussel filtration will affect the trophic interaction and distribution of contaminants.

CONCLUSIONS

Zebra mussels show different responses in bioprocessing when exposed to different algal concentrations and algal species.

1. For the zebra mussels fed Chlamydomonas reinhardtii, as the algal concentration increases, the filtering rate decreases and the pseudofeces increase. The algal assimilation efficiency is independent of algal concentration. Also, the total mass of algae filtered by the zebra mussels increases following the increased algal concentration, then it level off.

2. For the zebra mussels fed Chlorella vulgaris, the filtering rate decreases following increased algal concentration. Both the pseudofeces and the total algae filtered increase as the algal concentration increases. However, the algal assimilation efficiency does not vary with the algal concentration.

3. For the zebra mussels fed Ankistrodesmus falcatus, the pseudofeces and the algae filtered increase in response to the increased algal concentration. The filtering rate increases at the initial increasing algal concentrations, after reaching a maximum, the filtering rate decreases as the algal concentration increases.
CHAPTER III

ASSIMILATION AND TRANSFER OF HYDROPHOBIC CONTAMINANTS FROM INGESTED ALGAE BY ZEBRA MUSSELS, DREISSENA POLYMORPHA

INTRODUCTION

Hydrophobic contaminants such as polychlorinated biphenyls (PCBs) are families of chemicals having the characteristics of water-insolubility, persistence in the environment, and resistance to physical, chemical, and biological degradation (Swackhammer and Skoglund 1993). These properties facilitate bioaccumulation by aquatic organisms. Zebra mussels, recent invaders to the North American fresh water systems, have been reported to readily accumulate hydrophobic contaminants because of high filtering rates and high lipid content (Fisher et al. 1993; Bruner et al. 1994). For instance, the bioconcentration factors (BCFs) of benzo(a)-pyrene (BaP) at 4°C and 12°C are 197,619 and 85,666 respectively (Fisher et al. 1993). In addition to uptake of contaminants directly from water, zebra mussels accumulate hydrophobic contaminants from contaminated media such as algae and sediments (Bruner et al. 1994).
Furthermore, biodeposition of contaminated filtered particles was predicted to be a contaminant source to benthic organisms living in or near mussel colonies (Bruner et al. 1994). Therefore, zebra mussels play a potentially important role in contaminant cycling in aquatic ecosystems.

It was postulated that zebra mussels could influence contaminant cycling in two ways: 1) by direct contaminant transfer, in which contaminated zebra mussels may transfer contaminants from their bodies to their predators; or (2) by indirect contaminant transfer, where unassimilated contaminants in the mussel pseudofeces and feces may be transferred to benthic organisms, which ingest mussel fecal and pseudofecal materials. Both routes could ultimately lead to significant contaminant exposure to organisms at higher trophic levels.

Uptake of chemicals from water system by aquatic organisms depends on the bioavailability of the chemicals to the organisms (Spacie and Hamelink 1985). Freely dissolved contaminants in water diffuse across the surfaces of organisms, primarily the gills, much faster than the chemicals bound to suspended particles, which must desorb from particles before absorption by organisms. However, the accumulation of contaminants by mussels from contaminated ingested particles was shown to be an important route of long-term dietary exposure especially when the chemical concentrations in the water were low based on steady-state calculation (Bruner et al. 1994). Because hydrophobic contaminants are readily sorbed to organic-rich particles such as algae, the contaminant concentrations in water are often low. As a result, the uptake of contaminants from ingested
materials represents an important exposure route. In fact, a number of studies has verified that uptake of contaminants through dietary uptake is very important for the contaminant accumulation by aquatic organisms (Landrum et al. 1991; Dabrowska and Fisher 1993).

Contaminant assimilation efficiency (AE) is an important parameter for determining bioaccumulation potential from ingested particles and is measured as the ratio of the contaminants retained by the organisms to the total amount of contaminants ingested by the organisms. Accurate measures of contaminant transfer efficiency are required to improve the accuracy of bioaccumulation models (Thomann et al. 1992). In this study, zebra mussel contaminant AEs were measured from contaminant-spiked Chlamydomonas reinhardtii and Chlorella vulgaris. Three PCB congeners, 2,2',4,4'-tetrachlorobiphenyl (TCBP), 3,3',4,4',5-pentachlorobiphenyl (PCBP), 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP), and 1,1-dichloro-2,2-bis[4-chlorophenyl] ethylene (DDE) were used in the experiments. They are representatives of contaminants in fresh aquatic ecosystems such as the Great Lakes (Fitchko 1986). The different chemical properties of the four compounds such as partition coefficients should result in different contaminant AE (Muir and Yarechewski 1988; Bierman 1990).

In addition, algal species also affect accumulation of contaminants bound to algae because of the difference in their composition and digestibility, which determine the gut retention time and amounts of ingested contaminants.
*Chlamydomonas reinhardtii* is spherical and motile, and its cell wall contains no cellulose which makes it easier to digest (Brendelberger and Jürgens 1993). *Chlorella vulgaris* has a similar size and shape as *Chlamydomonas reinhardtii*, but is nonmotile, and has cellulose in its cell wall. Bioprocessing experiments (Chapter I) have demonstrated that zebra mussels prefer *Chlamydomonas reinhardtii* to *Chlorella vulgaris* based on the fact that more of the filtered algae are ingested and less pseudofeces are produced when mussels were fed *Chlamydomonas reinhardtii* compared to filtering a similar amount of *Chlorella vulgaris*. Therefore, if mussels are fed a similar amount of contaminated *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, more contaminants will be ingested when mussels are fed *Chlamydomonas reinhardtii*.

As mentioned above, the direct and indirect contaminant transfer by zebra mussels could greatly influence the contaminant cycling in aquatic ecosystems. Little work has been done on the role of zebra mussels in indirect contaminant transfer. The availability of contaminants in zebra mussel pseudofeces and feces to benthic organisms has been shown to serve as contaminant sources to benthic organisms. For instance, *Gammarus fasciatus*, a benthic amphipod, could assimilate contaminants from contaminated feces produced by zebra mussels (Bruner *et al.* 1994). In this study, the transfer of different contaminants to zebra mussel pseudofeces and feces was quantified using two algal species, *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, which were varied in
morphology and composition. These data help to refine the role of zebra mussels in the indirect contaminant transfer to the benthos.

MATERIALS AND METHODS

MATERIALS

Chemicals

\(^{14}\text{C-labeled } 2,2',4,4',5'-\text{tetrachlorobiphenyl (TCBP)}, \quad 3,3',4,4',5'-\text{pentachlorobiphenyl (PCBP)}, \quad 2,2',4,4',5,5'-\text{hexachlorobiphenyl (HCBP)} \text{ and } 1,1'-\text{dichloro-2,2-bis[4-chlorophenyl] ethylene (DDE) were obtained from Sigma Chemical company (St. Louis, Mo.). The specific activity of each chemical was } 12.6 \text{ mCi/mmol (TCBP), } 18.5 \text{ mCi/mmol (PCBP), } 21.2 \text{ mCi/mmol (HCBP) and } 12.7 \text{ mCi/mmol (DDE) respectively. Their purity is greater than } 97\% \text{ as determined by thin layer chromatography and radiometric analysis (Leversee et al. 1982).}

Organisms

Zebra mussels (Dreissena polymorpha) were collected by SCUBA divers from littoral zones in North Bay, Kelly's Island, Lake Erie. The collection season was from early spring to early fall. The mussels were kept cool and moist during transport to the Ohio State University, Columbus, Ohio. The mussels were held in 200-L aquariums filled with aerated carbon-filtered tap water at 12°C. A suspension of mixed diatoms (Oyster Diet B, Coast Seafood Company) or live algal mixture (Chlorella vulgaris, Chlamydomonas reinhardtii and
Ankistrodesmus falcatus) was fed to zebra mussels on alternative days (about 3.3g food per 1000 zebra mussels). Water quality in aquaria containing zebra mussels was monitored daily for temperature, pH and dissolved oxygen. Sixty percent of the water in the aquaria was changed weekly. To prevent the dissemination of veligers into local waterways, discarded water was treated with chlorine bleach (50 ppm) for 24 h before disposal. At least two weeks before the experiments, zebra mussels were transferred to incubators for acclimation to test conditions (temperature, pH and alkalinity [130mg/L as CaCO3 and hardness, 170mg/L as CaCO3]). Zebra mussels were considered to be suitable for use only when they could reattach to petri dishes within 48 h after severing their byssal threads.

Algae

Two species of algae (Chlorella vulgaris and Chlamydomonas reinhardtii) were used. The initial populations of each algal species were obtained from Carolina Biological Supply. Algal media were prepared according to Nichols and Bold (1965). Monocultures of each algal species were maintained in 9:1 Bold’s Basal Medium under a light regime of 16:8 hour (light: dark). The purity of the monoculture was checked weekly using a light-microscope.

METHODS

Media Preparation
The algae used in the high and low algal concentrations were spiked together to maintain the same chemical concentration. The algal concentration in algal culture was measured by a Coulter Counter Model ZBI (Hialeah, Florida). The data were used to determine the total amount of algal culture needed in the contaminant assimilation efficiency experiments. The required amount of algal culture was centrifuged for 30 min. at 2000 rpm, then the supernatant was decanted. The settling algal pellets were resuspended in 80-100 mL Hard Standard Reference Water (HSRW), spiked with a radiolabeled chemical and agitated overnight in the darkened incubator at 4°C.

On the day of the experiment, the spiked algae were centrifuged to remove the supernatant, then washed three times with HSRW to displace the chemicals loosely bound to the algae. After rinsing, the algal pellets were resuspended in 200 mL HSRW. Then, the algal cell concentration was measured with the Coulter Counter to determine the volume of algae needed for the high and low algal cell concentration experiments (for *Chlamydomonas reinhardtii*, the high and low algal concentration is about 8 ug ml\(^{-1}\) and 4 ug ml\(^{-1}\) respectively; for *Chlorella vulgaris*, the high and low algal concentration is about 5 ug ml\(^{-1}\) and 2.5 ug ml\(^{-1}\) respectively).

**Zebra Mussel Contaminant Assimilation Efficiency Experiments**

Previous experiments used the pulse-chase method of Luoma *et al.* (1992) to determine contaminant assimilation efficiency. In the pulse-chase experiment, organisms were fed with radiolabeled food for certain time, then the organisms
were transferred to clean water and fed with unlabeled food to “chase” the unassimilated materials from the gut. Because contaminant concentrations in the feces and pseudofeces were measured to estimate the contaminant transfer from algae to feces and pseudofeces, the pulse-chase method was not completely suitable, and was modified. Specifically, the chase period was eliminated to prevent the dilution of chemical concentration in the feces and pseudofeces by the unlabeled algae. Briefly, in the modified method, zebra mussels were fed with radiolabeled food for 2 h, and were transferred to clean water without feeding algae in the following 72 h, during which the mussels purged the contaminants by egesting them in the feces. Preliminary experiments have demonstrated that zebra mussels would assimilate most of the ingested algae and contaminants within 72 h.

On the day of the experiment, for both high and low algal concentration experiments (same chemical concentration), 8 zebra mussels were placed in each of the three replicate aquaria containing spiked algae. The fourth aquarium contained the same amount of spiked algae but without zebra mussels, and was used as a control group to monitor the settling of algae. At the beginning of the experiment ($T_0$), triplicate samples (50 mL) from each aquarium were filtered through preweighed 0.5 μm glass filters (Fisher Scientific, Pittsburgh, PA), then dried in the oven for at least 24 h at 60°C to determine algal mass density. Also, triplicate samples (15 mL) were taken to measure the radioactivity in water and algae (dpm mL$^{-1}$) using a Beckman LS 6000IC scintillation counter (14C efficiency > 95%) (LSC) with automatic quench control. After taking these samples, aquaria were placed in a darkened incubator at 22°C for 2 h.

After 2 h, aquaria were taken from the incubator. Triplicate samples from each aquarium were taken to determine the algal mass density and contaminant
concentration ($T_2$) in water and algae. The samples were processed as they were done at $T_0$. Then zebra mussels were transferred to HSRW without algae. The pseudofeces and feces produced during the 2 h exposure were collected separately with a pipette, filtered through preweighed 0.5 μm filters, aspirated at a rapid rate for 5 min., and dried in a dessicator for a couple of hours until steady dry weight was obtained. The contaminant mass in the pseudofeces and feces was determined by Liquid Scintillation Counting (LSC). Tissue solubilizer (0.5-1 mL) was added to each of the feces and pseudofeces samples before analyzing. After 30 min., the solubilizer was neutralized with acetic acid. Fifteen mL of scintillation cocktail (1000 mL dioxane, 100 g naphthalene, 5 g 2,5-diphenyloxazole) were added to each scintillation vial containing pseudofeces or feces before analyzing by LSC.

Seventy-two hours following the exposure, HSRW in the beakers containing mussels was changed every 24 h. The pseudofeces and feces were collected separately for each aquarium at 24 h, 48 h, and 72 h. They were dried, weighed, analyzed for contaminant measurements as described above.

**Mussel Contaminant Percent Assimilation Efficiency (%AE) Calculation**

The mussel contaminant percent assimilation efficiency for each contaminant is calculated using the chemical mass-balance equation:

$$%AE = \left[ \frac{(B-P-F)}{(B-P)} \right] \times 100$$

where B, P and F are the total chemicals mass filtered (μg), chemical mass(μg) in the pseudofeces and feces for eight zebra mussels respectively. The assumptions of the model are: (1) contaminants absorbed to algae have three potential fates: a) assimilation into tissues, b) expulsion in the feces, and c) expulsion with pseudofeces; (2) no chemicals desorb from the spiked algae during the 2 h exposure; (3) there is no significant elimination of contaminants
from pseudofeces and feces to the water; (4) 72 h is long enough for mussels to assimilate chemicals completely; and (5) the reduction of chemical mass in the exposure system is equal to the chemical mass filtered by the zebra mussels.

Data Analysis

Single-factor ANOVA (analysis of variance) F-test was employed to analyze significant differences for parameters such as zebra mussel contaminant assimilation efficiencies at different algal concentrations for each chemical. Also, Tukey’s multiple comparison was applied to further investigate the difference between individual variables. An alpha level of 0.05 was used in the data analysis as the level of significance.

RESULTS

Zebra Mussel Contaminant Assimilation Efficiencies from Chlamydomonas reinhardti and Transfer of Contaminants to the Feces and Pseudofeces

Contaminant Transfer From Ingested Algae to Feces and Pseudofeces

Zebra mussels cleared the ingested contaminants from their gut and passed the unassimilated contaminants to feces. The cumulative amount of TCBP, PCBp, HCBP and DDE eliminated via the feces during the depuration period is presented in Figs 10-13. For each contaminant, zebra mussels eliminated contaminants rapidly during the first 24 h and the total elimination approached a plateau by 72 h. Also, for each chemical, zebra mussels fed a high concentration of Chlamydomonas reinhardti expelled more contaminants in feces at every time interval than those fed a low concentration of Chlamydomonas reinhardti,
except for DDE. For DDE, mussels egested a similar amount of chemicals in feces when fed either a low or high concentration of contaminated *Chlamydomonas reinhardtii*.

Contaminant concentrations in the feces were in the range of 3.85 - 21.60 ng mg⁻¹, 8.74 - 25.29 ng mg⁻¹, 2.65 - 23.79 ng mg⁻¹ and 6.31 - 24.37 ng mg⁻¹ (dry weight) or TCBP, PCB, HCBP and DDE respectively, and they were always lower than that of ingested algae (Table 4). Also, the contaminant concentrations in the feces decreased with time. In general, the contaminant concentrations in the feces produced within 24 h were higher than those produced at 72 h (ANOVA, P<0.05).

When mussels were fed different concentrations of *Chlamydomonas reinhardtii* (same chemical concentration), contaminant concentrations in feces produced by mussels at a high concentration of *Chlamydomonas reinhardtii* were usually higher than those produced at a low concentration of *Chlamydomonas reinhardtii* at the same measured time (Table 4). This suggested that more contaminants were transferred to feces by mussels ingested higher concentrations of algae.

Zebra mussels produced pseudofeces only within 24 h after exposure to spiked *Chlamydomonas reinhardtii* (Table 5). Like that of feces, the contaminant concentration in the pseudofeces decreased with time for each chemical, and the contaminant concentrations in pseudofeces found at 24 h were lower than those of ingested algae. But, the pseudofeces contaminant
concentrations at 2 h were higher than that of ingested algae except for TCBP and PCBP-contaminated pseudofeces produced by mussels at the low algal concentrations.

Contaminant Percent Assimilation Efficiencies (%AE)

The %AEs were apparently independent of algal concentration, excluding TCBP (Figure 14). For TCBP, the contaminant %AE at the low concentration of *Chlamydomonas reinhardtii* was higher than the %AE determined at the high concentration of *Chlamydomonas reinhardtii* (ANOVA, P<0.05). Comparing contaminant assimilation efficiencies among the four chemicals at low and high algal concentrations respectively, PCBP assimilation efficiency was significantly lower than the contaminant %AEs of others (ANOVA, P<0.05), and contaminant %AEs of the other three contaminants were not significant from each other.

**Zebra Mussel Contaminant Assimilation Efficiencies from Chlorella vulgaris and Transfer of Contaminants to the Pseudofeces and Feces**

**Contaminants Transfer From Ingested Algae to Feces and Pseudofeces**

Zebra mussel gut clearance of TCBP, PCBP, HCBP and DDE after exposure to low and high algal concentrations of contaminated *Chlorella vulgaris* is illustrated in Figs 15-18. Compared with that of mussels fed contaminated *Chlamydomonas reinhardtii*, the elimination was much slower. But again, it approached a plateau by 72 h. At each time interval, zebra mussels expelled significantly higher amounts of chemicals in feces when fed higher
concentrations of *Chlorella vulgaris* than when fed lower concentrations of *Chlorella vulgaris* (ANOVA, P<0.05).

Algal contaminant concentrations were higher than fecal contaminant concentrations for all chemicals (Table 6). Although there are some outliers in the data sets, the tendency that contaminant concentration in the feces decreased with time was very consistent. For each chemical at low or high algal concentrations, the contaminant concentrations in the feces at 2 h and 24 h were usually significantly higher than that at 72 h (ANOVA, p<0.05).

The fecal contaminant concentrations also varied with the ingested algal concentrations for each chemical at different time intervals. Generally, at each time interval, mussels had lower fecal contaminant concentrations when fed a low concentration of *Chlorella vulgaris* than when fed a high concentration of *Chlorella vulgaris* (Table 6).

The contaminant concentrations in the pseudofeces produced when mussels were fed low and high algal concentrations for each chemical at different time intervals are illustrated in Tables 7-8. Unlike the mussels fed contaminated *Chlamydomonas reinhardtii*, which produced pseudofeces only within 24 h, mussels fed *Chlorella vulgaris* produced pseudofeces within 48 h after exposure to spiked algae. The contaminant concentrations in the pseudofeces was usually lower than that of ingested algae. Also, the contaminant concentration in the pseudofeces decreased with the time.
Contaminant percent assimilation efficiencies (%AE)

With the except of PCBP, the contaminant %AEs were independent of ingested algal concentration ( ANOVA, P>0.05 ) ( Figure 19.). %AEs among the four chemicals at low and high algal concentration were compared respectively. At low algal concentration, PCBP assimilation efficiency was significantly lower than the TCBP and DDE %AEs ( ANOVA, P<0.05 ), and contaminant %AEs of the other contaminants were not significant from each other. At high algal concentration, no significant differences existed among the four chemicals.

DISCUSSION

The uptake and assimilation of hydrophobic chemicals such as PCBs and DDT by aquatic organisms is well documented. Two models have been proposed to illustrate the process of assimilation of hydrophobic chemicals in the gut( Gobas et al. 1993 ). The first is the coassimilation model, which assumes that the assimilation of chemicals in the gastrointestinal tract ( GIT ) is through the coassimilation with lipids, namely, chemicals move cross the GIT with the lipid. The second model assumes that chemicals are assimilated from the gastrointestinal tract ( GIT ) by means of passive diffusion. That is the flux of the contaminants from food into the organisms is primarily governed by the driving forces of chemicals such as fugacity between the GIT and food. The two models of assimilation of contaminants differ in the mode of intestinal absorption
Figure 10. The cumulative TCBP in zebra mussel feces (n=8) collected after exposing to spiked Chlamydomonas reinhardtii. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).

Figure 11. The cumulative PCBP in zebra mussel feces (n=8) collected after exposing to spiked Chlamydomonas reinhardtii. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).
Figure 12. The cumulative HCBP in zebra mussel feces (n=8) collected after exposing to spiked *Chlamydomonas reinhardtii*. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).

Figure 13. The cumulative DDE in zebra mussel feces (n=8) collected after exposing to spiked *Chlamydomonas reinhardtii*. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).
Table 4. The transfer of contaminants from *Chlamydomonas reinhardtii* to feces. Numbers in parenthesis are standard errors. H and L represent high and low algae concentration respectively. Within a row, values with the same letter (s) are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Concentration in algae (ng mg⁻¹)</th>
<th>Chemical Concentration in feces (ng mg⁻¹)</th>
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<td></td>
<td></td>
<td>2h</td>
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</tr>
<tr>
<td>L</td>
<td>29.09</td>
<td>8.74&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.45)</td>
<td>(1.89)</td>
</tr>
<tr>
<td>HCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>26.85</td>
<td>23.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.07)</td>
<td>(1.62)</td>
</tr>
<tr>
<td>L</td>
<td>23.60</td>
<td>20.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.03)</td>
<td>(1.37)</td>
</tr>
<tr>
<td>DDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>33.50</td>
<td>24.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(2.43)</td>
<td>(1.19)</td>
</tr>
<tr>
<td>L</td>
<td>29.07</td>
<td>22.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(1.98)</td>
<td>(0.46)</td>
</tr>
</tbody>
</table>

Value with * is excluded from the analysis.
Table 5. The transfer of contaminants from *Chlamydomonas reinhardtii* to pseudofeces. Numbers in parenthesis are standard errors. H and L represent high and low algae concentration respectively.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Concentration in Algae (ng mg⁻¹)</th>
<th>Chemical Concentration in Pseudofeces (ng mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2h</td>
<td>24h</td>
</tr>
<tr>
<td>TCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>22.05</td>
<td>22.24(1.39)</td>
</tr>
<tr>
<td>L</td>
<td>23.56</td>
<td>15.76(1.25)</td>
</tr>
<tr>
<td>PCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>27.69</td>
<td>36.92(2.06)</td>
</tr>
<tr>
<td>L</td>
<td>29.09</td>
<td>14.95(1.90)</td>
</tr>
<tr>
<td>HCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>26.85</td>
<td>30.53(2.24)</td>
</tr>
<tr>
<td>L</td>
<td>23.60</td>
<td>29.52(0.63)</td>
</tr>
<tr>
<td>DDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>33.50</td>
<td>46.08(4.53)</td>
</tr>
<tr>
<td>L</td>
<td>29.07</td>
<td>37.71(4.30)</td>
</tr>
</tbody>
</table>
Figure 14: Comparison of four contaminant %AEs of zebra mussels at two different contaminant concentrations of C. renardii based on the chemical mass-balance. For each chemical, values with the same letter are not significantly different (P>0.05).
Figure 15. The cumulative TCBP in zebra mussel feces (n=8) collected after exposing to low concentration of spiked *Chlorella vulgaris*. Vertical bars represent standard errors.

Figure 15a. The cumulative TCBP in zebra mussel feces (n=8) collected after exposing to high concentration of spiked *Chlorella vulgaris*. Vertical bars represent standard errors.
Figure 16. The cumulative PCBP in zebra mussel feces (n=8) collected after exposing to spiked *Chlorella vulgaris*. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).

Figure 17. The cumulative HCBP in zebra mussel feces (n=8) collected after exposing to spiked *Chlorella vulgaris*. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).
Figure 18. The cumulative DDE in zebra mussel feces (n=8) collected after exposing to spiked *Chlorella vulgaris*. Vertical bars represent standard errors. (L and H represent low and high algal concentration respectively).
Table 6. The transfer of contaminants from *Chlorella vulgaris* to feces. Numbers in parenthesis are standard errors. H and L represent high and low algae concentration respectively. Within a row, values with the same letter (s) are not significantly different (p>0.05).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Concentration in algae (ng mg$^{-1}$)</th>
<th>Chemical Concentration in feces (ng mg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2h</td>
<td>24h</td>
</tr>
<tr>
<td>TCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>17.56</td>
<td>3.99$^a$</td>
</tr>
<tr>
<td>L</td>
<td>16.89</td>
<td>1.48$^a$</td>
</tr>
<tr>
<td>PCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>25.24</td>
<td>8.97$^a$</td>
</tr>
<tr>
<td>L</td>
<td>27.76</td>
<td>9.69$^a$</td>
</tr>
<tr>
<td>HCBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>20.55</td>
<td>9.92$^a$</td>
</tr>
<tr>
<td>L</td>
<td>20.33</td>
<td>5.04$^a$</td>
</tr>
<tr>
<td>DDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>26.30</td>
<td>11.65$^a$</td>
</tr>
<tr>
<td>L</td>
<td>24.88</td>
<td>8.15$^{ab}$</td>
</tr>
</tbody>
</table>
Table 7. Transfer of contaminants from *Chlorella vulgaris* (low algal concentration) to pseudofeces. Numbers in parenthesis are standard errors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical concentration in algae (ppm)</th>
<th>Chemical concentration in pseudofeces (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h</td>
<td>48h</td>
</tr>
<tr>
<td>TCBP</td>
<td>16.89</td>
<td>2.41 (0.33)</td>
</tr>
<tr>
<td>PCB BP</td>
<td>27.76</td>
<td>9.28 (1.25)</td>
</tr>
<tr>
<td>HCBP</td>
<td>20.33</td>
<td>8.03 (0.46)</td>
</tr>
<tr>
<td>DDE</td>
<td>24.88</td>
<td>11.46 (1.65)</td>
</tr>
</tbody>
</table>

Table 8. Transfer of contaminants from *Chlorella vulgaris* (high algal concentration) to pseudofeces. Numbers in parenthesis are standard errors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical concentration in algae (ppm)</th>
<th>Chemical concentration in pseudofeces (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2h</td>
<td>24h</td>
</tr>
<tr>
<td>TCBP</td>
<td>17.56</td>
<td>9.36 (0.55)</td>
</tr>
<tr>
<td>PCB BP</td>
<td>25.24</td>
<td>16.80 (4.52)</td>
</tr>
<tr>
<td>HCBP</td>
<td>20.55</td>
<td>14.21 (0.55)</td>
</tr>
<tr>
<td>DDE</td>
<td>26.30</td>
<td>23.08 (1.67)</td>
</tr>
</tbody>
</table>
Figure 19. Comparison of four contaminant %AEs of zebra mussels at two different *C. vulgaris* concentration based on the chemical mass-balance. For each chemical, values with the same letter are not significantly different (P>0.05).
and also differ in the site of biomagnification. Although several authors have pointed out the importance of lipid cotransport in the dietary uptake of hydrophobic contaminants (Vetter et al. 1985), the passive diffusion of hydrophobic chemicals in the GIT has been considered to be the major route for the assimilation of hydrophobic contaminant by organisms (Gobas et al. 1993).

The second model of assimilation of hydrophobic chemicals in the GIT is explained by fugacity theory. Fugacity is equivalent to chemical activity or chemical potential. The chemicals diffuse from high fugacity to low fugacity until equilibrium is reached, in which fugacity in all phases is equal (Mackay et al. 1982). In the GIT, food digestion and absorption would result in the increasing fugacity of chemicals above that of consumed food (Gobas et al. 1993). Consequently, the gradient of fugacity would serve as a pump for the assimilation of chemicals from food to the GIT.

It is obvious that uptake and assimilation of hydrophobic chemicals from ingested food involves the desorption of chemicals from ingested food before they are assimilated. Thus, assimilation of hydrophobic chemicals from ingested food is influenced by a variety of factors, which determine the desorption rate of chemicals. Accordingly, the contaminant assimilation efficiency that describes the assimilation would also be affected by these factors determining desorption rate. In addition to biological and physical parameters such as the feeding type and contaminant gut retention time (Clark and Mackay 1991; Harkey et al. 1994; Fisher 1995), compound chemical characteristics such as octanol/water
partition coefficients and molecular weight (Niimi and Oliver 1988; Bierman 1990) would also affect the contaminant assimilation efficiencies.

For hydrophobic contaminants, an important parameter affecting assimilation efficiency is octanol/water partition coefficient ($K_{ow}$). The higher the chemical’s partition coefficient, the stronger the chemical affinity to the ingested food. Thus, as contaminant $K_{ow}$ increases, fewer contaminants are desorbed from the food and assimilated by organisms, and the contaminant assimilation efficiencies decrease. Many studies have proven that assimilation efficiencies are negatively correlated to compound partition coefficients ($K_{ow}$) when $\log K_{ow} < 7$ (Muir and Yarechewski 1988; Bierman 1990). In the contaminant AE experiments, although significant differences in contaminant assimilation efficiencies existed among different chemicals, for example, PCB%AE was significantly lower than the others, no correlation between compound partition coefficient and contaminant assimilation efficiencies was found. This may result from the narrow range of partition coefficients ($\log K_{ow} = 6-7$) for the compounds used, while, other researchers used a wider range of $K_{ow}$ ($\log K_{ow} = 4-7$). It is not clear why PCB%AE is lower than others. Possible reasons may be related to the health of zebra mussels. PCB was the last chemical tested for one species of algae. At that time, because of the long holding time in lab, zebra mussels were not as healthy as those used to test for other chemicals. Thus, physiological factors that were not measured may have contributed to the results.
The contaminant %AEs were calculated based on the chemical mass-balance model, and they were independent of the two algal concentrations. This trend was similar to the algal assimilation efficiencies described in Chapter I. As described above, the uptake of chemicals from ingested food occurs in the GIT. The fraction of the chemicals transferred from ingested food to the organisms would depend on the chemical fugacity in the food and organisms (Clark and Marckay 1991). According to the fugacity model, the steady-state fugacity in organisms will increase as the chemical fugacity in food increases. The relationship between the fugacity in the organisms ($f_F$) and in the food ($f_A$) would be described as:

$$f_F = \frac{D_A f_A}{(D_w + D_R + D_E + D_D)}$$

where $D_A$, $D_w$, $D_R$, and $D_E$ are mass transport parameters for exchange with water at the food uptake, gills, metabolism, elimination to the feces respectively (mol/Pa.h). $D_D$ represents growth dilution. Thus, doubling the food concentrations will double the organism steady-state contaminant concentrations, which implied that more contaminants will be assimilated in the gut when organisms ingest more contaminated food assuming that none of the transfer processes is affected. However, the contaminant assimilation efficiencies are not only controlled by the chemical fugacity, but are also dependent on bioprocessing parameters when zebra mussels are faced with different algal concentration. Mechanically, the passage of food through the GIT is an important mechanism for the elimination of contaminants and can be accelerated by ingestion of more
food and egestion of more feces (Gobas et al. 1993). For instance, Clark and Mackay (1991) reported that contaminant assimilation efficiencies decreased when an identical amount of chemical was fed to guppies along with a large food volume. The reduction in contaminant %AE was ascribed to the rapid egestion rate of feces. In the experiment, at high algal concentration, zebra mussels not only ingested more contaminants, but also egested more feces, which may counterbalance the possible high contaminant %AE. On the other hand, the total amount of chemicals egested in the feces was minimal compared with the total contaminants ingested in the gut. Thus, the contaminant %AEs determined by the chemical mass-balance model could not show the difference between low and high algal concentrations.

If comparing the mussel contaminant %AE between the two species of algae spiked with same chemicals, %AEs were independent of algal species. Again, the amount of contaminants in the feces is minimal compared with the total amount of chemicals ingested by the mussels, so the contaminant assimilation efficiencies would not differ with algal species.

Comparing the algal assimilation efficiencies measured in the chapter II, contaminant assimilation efficiencies are higher than the algal assimilation efficiency. It suggests that contaminants are not coassimilated with food, but diffuse from food to organisms (Gobas et al. 1993). Also, it indicates that the contaminants are not proportionally assimilated with algae, and contaminants are
more efficiently assimilated than algae. Otherwise, the algal assimilation efficiencies would be equal to the algal assimilation efficiency.

For identical chemicals, %AE values measured in our studies were significantly higher than those determined in the pulse-chase method (Bruner et al. 1994). Besides some uncontrolled factors such as lipid content of zebra mussels in the two experiments, the differences in the two methods may also yield a differences in the contaminant %AE. In the pulse-chase method, unlabeled algae were fed to zebra mussels in the chase periods to chase the unassimilated materials including contaminants from gut. While, in the modified pulse-chase method, no algae were fed to the zebra mussels to chase the unassimilated contaminants in the gut, which implied that contaminants would stay longer in the gut. Also, the “chase” period in the modified pulse-chase experiments was 72 h, and the chase period in the pulse-chase experiments was only 48 h. The different time either the contaminant retention time in gut or measure time in the two experiments may be attributed to the difference in %AEs.

In fact, gut passage time (GPT) or the time required for the ingested particles to pass through or clear from the gut has been considered to be a key parameter affecting assimilation efficiency (Kolfoed et al. 1989). Connell (1989) suggested that the transfer efficiencies of chemicals from food would differ for the same bioconcentration process in different times over which the transfer efficiency is measured. In addition, Klump et al. (1987) reported that
the HCBP assimilation efficiency increased with the gut clearing time. In a study of assimilation of Selenium (\(^{75}\)Se) by *Macoma balthica*, the organism assimilation efficiency of Se increased during the first 10 h, then leveled off, which suggested that \(^{75}\)Se was more efficiently assimilated in the later digestion process than in the initial hours (Luoma *et al.* 1992).

In addition to using chemical mass-balance method to calculate contaminant %AEs, two other methods have been involved to measure contaminant %AEs in other research, both of them use tracers. One uses dual radioactive tracers, the other use organic carbon as a tracer. For the dual tracers, one tracer is assimilated (\(^{14}\)C-labeled compound) by organisms, the other could not be assimilated such as \(^{51}\)Cr and Polydimethylsiloxane (PDMS) (Klump *et al.* 1987; Lopez and Elmgren 1989; Kukkonen and Landrum 1995). If the non-assimilated and the assimilated tracer are randomly bound to particles, and also they are indiscriminately ingested by organisms, contaminant %AE could be calculated by comparing the ratio of the activities of the two tracer in the feces and ingested particles. Using this method, contaminant %AEs have been measured for some benthic organisms such as oligochaetes (Klump *et al.* 1987). However, the method does not work if the organisms violate the assumptions for using the dual-tracer method. For instance, organisms may selectively ingest particles containing \(^{14}\)C over those containing non-assimilation tracer, which may lead to the failure to correctly measure %AE (Lydy and Landrum 1993). For the organic tracer method, contaminant %AEs are calculated by measuring the
carbon-normalized contaminant concentrations in the feces and ingested particles. This method has been used to measure the contaminant %AE for benthic organisms ingesting sediment (Lee et al. 1990; Lydy and Landrum 1993; Kukkonen and Landrum 1995). However, this approach requires an estimation of the carbon organic assimilation and the feeding selectivity. Some benthic organisms preferentially ingest organic-rich sediment to organic-poor sediment (McMurtry et al. 1983), and the organic rich sediment may have higher contaminant concentration than those organic-poor sediment. Therefore, it is possible that the feces have higher contaminant concentrations than the ingested sediment if the contaminant concentrations in the ingested sediment are calculated based on the bulk sediment. Use of a selectivity index in the calculation could correct for the selection of organic carbon-rich sediment by organisms (Lee et al. 1990; Lydy and Landrum 1993; Kukkonen and Landrum 1995).

The contaminants found in feces and psuedofeces support the hypothesis that the zebra mussels could pass the unassimilated contaminants to feces and pseudofeces. Over time, contaminant concentrations in the feces decreased. The trend may be ascribed to the digestion process and contaminant gut retention time, which would affect the assimilation of nutrients as well as accumulation of contaminants (Borchardt 1985; Spacie and Hamelink 1985). Bivalves have two digestive processes: intestinal digestion and glandular digestion (Morton 1983). Intestinal digestion is an extracellular digestion process, in which the ingested
matter may cross the membranes of the stomach and intestine after being
degraded extracellularly by digestive enzymes (Widdows et al. 1979). Glandular
digestion is the subsequent step to intestinal digestion, in which the finer particles
are further sorted after intestinal digestion and sent to the tubules of the digestive
glands, where they are digested intracellularly by digestive cells. Although the
relative importance of the intestinal digestion and glandular digestion is not well
understood, they are known to affect the uptake of trace metals by bivalves

Glandular digestion breaks down food more completely than intestinal
digestion. In addition, the food digested by the glandular digestion may stay in
the gut longer than the food just digested by the intestinal digestion. Therefore,
more contaminants would diffuse from food to GIT if they experienced both
digestive processes. In our experiment, feces were collected at different intervals
during gut clearing period. Visual inspection of the feces indicated that some
difference existed in the feces collected at different sampling times. Feces
produced within 24 h were usually green and uncompacted, while, the feces
produced from 24 h to 72 h were dark-green and more compacted. It is very
possible that the feces produced at different times were the results of different
digestive processes. Consequently, the degree of assimilation of contaminants
could change over time. The more time the food retained in the gut, the more
contaminants would diffuse to organisms and were assimilated, and the lower the
chemical concentrations in the feces.
Algal cell concentrations also affected contaminant concentrations in the feces. The contaminant concentrations in the feces produced by the zebra mussels fed low algal concentrations were lower than those of mussels fed high algal concentrations. As described above, the uptake of chemicals from ingested food occurs in the GIT. The gut passage time (GPT) would affect the contaminant assimilation. The more GPT, the more contaminants would diffuse from food to GIT. In the experiment, mussels egested more contaminants in feces and the produce of feces was faster at higher algal concentration than that at low algal concentrations. So, the algae may reside for a short in the gut before they are egested as feces compared to the feces produced at low algal concentrations. This may result in the higher contaminant concentrations in feces when mussels were fed high algal concentration.

At high algal concentration, there was a greater percent pseudofeces produced as demonstrated in Chapter II. Contaminants in the pseudofeces can transfer contaminants to benthic organisms living in mussel colonies. Therefore, the quantitative measurement of chemicals in pseudofeces is very important for ecological risk assessment. The chemical concentrations in pseudofeces were usually lower than that of the ingested algae, excluding the pseudofeces produced by the zebra mussels during the 2 h exposure when fed high concentrations of Chlamydomonas reinhardtii and low concentration of DDE and HCBP contaminated Chlamydomonas reinhardtii. In this instance, the chemical concentrations in the pseudofeces were higher than that of ingested algae.
Because pseudofeces are not digested, they should have had similar chemical concentrations to that of the ingested algae. The observed reduction of the chemical concentration in the pseudofeces may be due to the dilution of contaminant concentration by the formation of mucous, or the desorption of chemicals from the pseudofeces to water. Although the amount of mucous wrapping pseudofeces has not been quantified, it is likely that it would increase the mass of pseudofeces and thus decrease the measured chemical concentration in the pseudofeces. However, the desorption of chemical from pseudofeces to water is likely to be an important factor that affects the chemical concentration in the pseudofeces. Zebra mussels were held in the HSRW after exposure to the spiked algae. Pseudofeces produced during this period had significantly higher contaminant concentrations than that of water. As a result, the gradient of the chemical concentration would facilitate the desorption of chemicals from the pseudofeces to water.

It is not clear why the pseudofeces in some cases had higher chemical concentrations than that of ingested algae. Only Reeders and Vaate (1992) have reported that zebra mussel pseudofeces were more polluted than ingested particles. A possible reason may be that the pseudofeces produced within the 2 h exposure to spiked algae may adsorb some chemicals from water. Pseudofeces were wrapped in mucous, and this may change the surface area and other surface characteristics. These parameters are very important in determining the absorption ability. Another possible explanation for higher contaminant
concentrations in pseudofeces may result from the selectivity for less contaminated particles by zebra mussels. Zebra mussels are reported to produce less pseudofeces when ingesting non-toxic form of *Anabaena* than when ingesting same amount of toxic form of *Anabaena*, which is made by adding cyanobacterin, a natural inhibitor, to *Anabaena*, and the phenomenon may result from that zebra mussels could recognize the taste of simulated toxic bluegreens and expel them as pseudofeces (McNaught and DeSorcie 1993). In our experiments, it is possible that contaminants are not evenly distributed among the algae because of the age, size, lipid content. Therefore zebra mussels may selectively rejected those high contaminant concentrated algae as pseudofeces due to the chemical stimulation. As a result, the contaminant concentration in pseudofeces may be higher than those of ingested algae.

CONCLUSIONS

In summary, zebra mussels passed unassimilated contaminants to pseudofeces and feces following ingestion of contaminated *Chlamydomonas reinhardtii* and *Chlorella vulgaris*. The assimilation and transfer of hydrophobic contaminants by zebra mussels have significant ecological effects, that is, zebra mussel not only assimilate contaminants into their body, but also pass the unassimilated contaminants to feces and pseudofeces, which provide contaminant sources to benthic organisms.
1. The contaminant concentration in the feces varied with time for each chemical and each species of algae, and they decreased with time. Also, at each time interval, the mussels fed low algal concentrations had lower contaminant concentrations in feces than those of mussels fed high algal concentrations.

2. Contaminant concentrations in the pseudofeces were usually lower than that of ingested algae.

3. The contaminant %AEs based on the chemical mass-balance were independent of algal species and algal concentrations.
LIST OF REFERENCES


Sea Grant. 1995. Sea grant zebra mussel report. An uptake of Research and Outresearch.


