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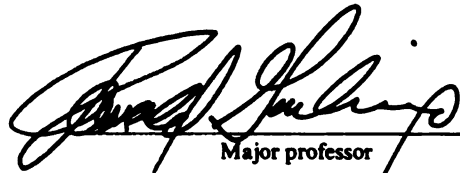
Food Web Dynamics and Dietary Habits of Freshwater
Unionidae: Implications for Captive Management
of a Decling Resource

presented by

Susan Jerrine Nichols

has been accepted towards fulfillment
of the requirements for

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Major professor

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**FOOD WEB DYNAMICS AND DIETARY HABITS OF FRESHWATER
UNIONIDAE: IMPLICATIONS FOR CAPTIVE MANAGEMENT OF A DECLINING
RESOURCE.**

By

Susan Jerrine Nichols

A DISSERTATION

**Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of**

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

1999

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ABSTRACT

FOOD WEB DYNAMICS AND DIETARY HABITS OF FRESHWATER UNIONIDAE: IMPLICATIONS FOR CAPTIVE MANAGEMENT OF A DECLINING RESOURCE.

By

Susan Jerrine Nichols

North America's Unionidae are fast approaching extinction due to a number of factors such as habitat degradation and proliferation of exotic species such as the zebra mussel (*Dreissena polymorpha*). Current conservation strategies have been marginally successful in part due to a lack of information about feeding habits and dietary needs of these filter-feeding bivalves. In this series of experiments, we have examined the feeding habits and trophic level interaction of a number of unionid species both in the field and in the laboratory. First, we first used stable isotope techniques to determine the diet of unionids under field conditions and then had this diet analyzed for nutritive content. We then looked at several potential mechanisms involved in food capture and in partitioning of food resources among various unionid species. This information was then applied to the development of a captive management protocol that proved useful for a number of species.

The first set of experiments compared food resource partitioning and trophic level relationships between unionid species in a detritus-dominated river and an algal-dominated lake using biochemical analyses, gut contents, and stable isotope ratios. The $\delta^{13}\text{C}$ values showed that all species from river and lake used bacterial carbons, not algal carbons, as their main carbon source. Algae did provide key nutrients such as vitamins A, D, and phytosterols that were bioaccumulated in the body tissues of all species. The $\delta^{15}\text{N}$ ratios that unionid

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species function as both primary consumers, omnivores, and possibly secondary consumers.

In the second experiments, we investigated the possibility that symbiotic microbial fauna was responsible for the slight differences in food assimilation among some unionid species. However, endemic microbial fauna were not a factor in food resource partitioning.

All unionid species from all habitats contained the same endemic microbe, which could only digest starch. Cellulolytic and chitinolytic microbes were transient, present only when the unionids were feeding, and were habitat, not species specific.

The third experiments focused on food capture mechanisms and showed that unionids capture benthic as well as planktonic food resources. Benthic feeding was accomplished through water currents generated from within the mantle cavity that moved food into the mantle cavity through non-siphon areas. Pseudofecal release was also more variable than expected, released from the inhalant siphon, and from anterior and middle mantle edges. Pseudofeces that collected at the base of the shell were accessible as a food resource and potentially represent a food storage mechanism.

Adult unionids are difficult to maintain in captivity and the commonly used live algal feeds do not meet nutritional needs resulting in high mortality after several years. In our final experiments, we applied information regarding field dietary preferences, nutrient bioaccumulation, and endosymbionts and tested a number of diet formulations (non-live algae) on several species of unionid adults. A number of diets supported growth and short-term survival of less than 3 years; a few supported reproductive efforts. The fastest growth and best survival over the test period was seen on an experimental diet that blended FPOM, dried algae, and nutrients (cholesterol and vitamin B12) accumulated by field animals.

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ACKNOWLEDGEMENTS

The U. S. Fish and Wildlife Service, the National Biological Survey, and the U. S. Geological Survey provided primary support for this research.

I am indebted to other colleagues and coworkers for their assistance in this work including Jeff Allen, Glen Black, and Dr. Douglas Wilcox of the U.S. Geological Survey; Drs. Thomas Dietz, John Lynn and Harold Silverman of Louisiana State University, and Jon Amburg and Steve Hart of the Department of Fisheries and Wildlife, Michigan State University.

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INTRODUCTION

Statement of Problem

Unionid mussels (freshwater clams) are one of the most endangered groups of animals in North America (Williams et al. 1993). North America has the largest diversity of unionids in the world (Metcalf-Smith et al. 1998), and most of these are located in the southeastern region of the United States. When compared to historical populations, many streams in eastern North America now possess depauperate mussel faunas. Williams et al. (1993) listed 297 species of native freshwater mussels in the United States and Canada. Of these, 213 species (71.7%) are considered endangered, threatened, or of special concern. Many of these species, 51 in the United States, are listed as endangered, and more are under review (Williams et al. 1993).

Decreasing unionid populations suggest that we are not protecting our aquatic resources adequately (Nalepa et al. 1991, Schloesser and Nalepa 1994). Unionids face a variety of threats including siltation, sedimentation, channelization, heavy metals, radionuclides, pesticides, human and feed lot wastes, mining wastes, acid runoff, and other contaminants (Schloesser and Nalepa 1994). These threats have been implicated as factors responsible for reducing the numbers and kinds of freshwater mussels (Fuller 1974, Marking and Bills 1980, Schloesser et al. 1996). Currently, agricultural and urban runoff remains as major non-point sources of nutrient enrichment and sediment load into surface waters (e.g., Justic et al. 1992, Strayer et al. 1999). With the increased spread of exotic species (i.e., the zebra mussel Figure 1), the unionids are in jeopardy of extirpation.

Figure 1. Native freshwater unionid biofouled by the exotic zebra mussel, *Dreissena polymorpha*. This biofouling results in the death of the unionid.



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During the past 20 years, state and federal agencies have increased efforts to develop management tools in an attempt to mitigate the continuing decline and extirpation of North American freshwater Unionidae, particularly at the community level. The most commonly used conservation strategy to date has been relocation, where whole unionid communities are moved into new habitats, either in other water bodies or within their native systems. With few exceptions, these unionid relocation efforts have failed. It has been estimated that over 50% of the 90,000 native clams moved in the past few years died within the first year in their new habitat (Cope and Waller 1995). A second conservation strategy has focused on captive management through aquaculture. Efforts to culture juvenile mussels of many species through the period of metamorphosis have been very successful for many species (e.g., Gatenby et al. 1996; Gatenby et al. 1997), but attempts to maintain adult unionids in captivity more than 2-3 years have generally failed (e.g., Gatenby et al. 1999). One major problem hindering conservation efforts for both field and captive populations is the lack of information on unionid dietary habits and requirements.

The primary objectives of my research were to: in Chapter 1, determine the food web dynamics of a multi-species unionid community under natural conditions; in Chapter 2, to test various hypothesis on mechanisms for resource partitioning among these species; in Chapter 3 to determine the nutritive component of unionid food resources; and finally, to use this information to develop a captive diet and maintenance program for adult unionids.

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North American Unionids

Freshwater native clams are the most endangered animal group in North America. Fresh water clams are found on every continent except Antarctica, but the greatest diversity of these freshwater mussels, approximately 270 species, occurs in North America. This group of mussels, belonging to the family Unionidae, has a number of common names such as freshwater pearly mussels, native freshwater mussels, freshwater clams, naiads, or unionids. Over the past century, native clam species have suffered severe declines in diversity and abundance due to human impacts on aquatic habitat, commercial harvesting, and the introduction of carp, water pollution, and the recent invasion of zebra mussels (*Dreissena polymorpha*). A frightening 72% of our 270 native mussel species are listed as extinct, endangered, threatened, or of special concern. In contrast, the Nature Conservancy considers only 13% of birds and mammals to be imperiled.

Unionids live in every type of river and lake and are easily recognized by their hinged shell. The shape size, thickness, and color of the shell varies greatly between species, but in all unionids, the shell forms a protective hinged box, completely covering the soft body of the clam. The shell is composed of three layers: a brownish outer skin, or periostracum, a middle rough calcareous layer called the prismatic layer, and a smooth inner layer, the nacre. The nacre is often colored deep pink or purple and is called mother-of-pearl. The shell can be large, with records of animals 1 m in length reported from the Mississippi River in the early 1900's. Typically, most unionids are less than 25 cm in length.

These animals are found half-buried in the mud or gravel. They orient themselves so that their head and foot are in the mud, and their posterior end is up in the water column.

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Unionids can crawl and change locations, but they are slow, capable of moving less than 3 m/day. Most remain fairly stationary unless some habitat stress occurs.

Unionids are long-lived animals, reaching ages greater than 100 years and many species do not even become sexually mature until they are 8-10 years of age. Sexes are usually separate, but if a unionid is isolated from a member of the opposite sex, it can turn into a hermaphrodite, both male and female, and fertilize its own eggs. Otherwise, male mussels release their spermatozoa into the water and the females filter the sperm from the water, and fertilization is inside the shell of the female, but technically outside of her body. The female sheds eggs into the cavity inside the shell, the mantle cavity, and fertilization occurs here. Female mussels brood their young from the egg to the larval stage in their gills. In the spring, or summer, depending on species, they expel the larvae, called glochidia, into the water where the larvae attach to the gills or fins of an appropriate fish host in order to become encysted and complete their metamorphosis to the juvenile stage. Unionid larvae are limited to the type of fish they can use as hosts—only certain species are acceptable.

Since unionids are not very mobile, some method of attracting the right type of fish to the female clam has to be used. The females of some species have developed a fake lure, that looks like an item the fish would like to eat, and the clam works this lure so that it looks alive. Once a fish tries to eat this lure, the female unionid releases glochidia that in turn attach to the fish. After a period of attachment to the fish ranging from 1 to 25 weeks, depending on the type of unionid, the juvenile mussel detaches from its host and falls to the substrate to complete its development into a free-living adult. Some species may successfully use a variety of fishes, but the majority are thought to be host-specific. It is partly because of this dependency on the glochidial host that mussels are so sensitive to

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environmental disturbances. Not only are unionids threatened by disturbances that impact them directly, but also by those altering the host fish populations. In several cases, mussel species have become functionally extinct due to the decline or disappearance of host fish species.

The importance of unionids to the ecosystem relates directly to their feeding habits and their size. Unionids are nature's water filtration plants. They are filter feeders that process up to 40L of water per clam per day. As all this water moves through the unionid, finger-like structures called cilia, located on the gills, catch and remove any particles in the water column. The unionid sorts through these particles and eats some material and ejects other particles in the form of pseudofeces. Pseudofeces are particles that have been drawn inside the shell of the clam, but have not been digested or ingested into the gut. Unionids feed on many different items, but bacteria such as *E. coli*, and the types of blue-green algae that causes taste-and-odor problems in human water supplies are favored foods. Since unionids are one of our largest freshwater invertebrates, they dominate the benthic biomass in many systems. At one time, unionids occurred in densities of over 100/m² in many watersheds in North America, and as such their importance in keeping waters clear cannot be underestimated (e.g., Strayer et al. 1999).

The vulnerability of native unionids to human-derived impacts can be attributed to a number of factors. Since they are long-lived filter feeders that are relatively stationary, they tend to accumulate and concentrate toxins over many years. As such, many unionid communities have been poisoned due to industrialization of surrounding landscape that occurred in the 1950's and 1960's. Unionids also suffer from dredging activities, since they cannot move away from the dredge and are often killed by the damming of rivers to create

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lakes. Nor do unionids respond well to chlorine residues or the chemicals used to kill aquatic weeds in the lakes or on lawns along the shoreline. Further problems have been caused by the required relationship between unionid larvae and fish. Many unionid populations have become functionally extinct because their fish host, such as the snail darter, has become rare.

Population declines have also occurred through direct predation and harvesting of unionids by humans. Native clams were frequently used as food items by Native American tribes and early European settlers. The discovery of a large pearl in the 1850's in a clam in Ohio started a massive "pearl rush" reminiscent of the California gold rush. Hundreds of thousands of unionids were destroyed in what proved to be a fruitless search for pearls. While all unionids can form pearls in response to some irritating sand grain or parasite that gets trapped inside the shell by the nacre, most such pearls are chalky and soft, and of no value. Just as the "pearl rush" died down, a commercial button industry formed to make buttons from the nacre of the shell. By 1912, there were 200 button factories in the United States, harvesting 60 thousand tons of unionid shell per year, representing an estimated 600 million live animals (Madson, 1987). Shell was so valuable that harvesters were known to use cannon to blow competitors' boats out of the water. This button harvest continued unabated until the 1920s with fewer and fewer live unionids found each year. The development of plastic buttons halted the harvest of shell for buttons. However, after World War II a new threat appeared, with the use of unionid shell as part of the cultured pearl industry. The rise in popularity of cultured pearls required the use of some sort of core for the pearl oyster to use as a base for the pearl. Beads cut from the shell of certain unionid species proved most effective for this purpose. At this time, any cultured pearl sold in the

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world has as its core, a piece of North American unionid shell. Further decline in unionid populations has led to widespread poaching of animals from areas not open to harvest. In response to this poaching activity, it is now against the law to possess a live unionid or a unionid shell in the state of Michigan.

Conservation efforts to help these animals are not limited to just state and federal agencies. Public education will be key to establishing effective conservation measures. Public interactions with unionids are limited, but usually deleterious to the animal. Education efforts need to focus on encouraging people to leave unionids alone: unionids do not live long in aquaria or garden pools; they cannot survive if removed from the water as they are incapable of crawling back into the water from shore; and clams are sensitive to lawn herbicides and pesticides which should not be applied to shore. One question that is constantly asked by the general public is if unionids can be eaten. Unionids are no longer safe to eat unlike the salt-water clams, due to the accumulation of decades of pollutants including PCBs, dioxins, and fecal coliform bacteria in the freshwater animals. Furthermore, as mentioned earlier, it is now against the law to harvest, kill, or possess a live unionid or a unionid shell in the state of Michigan. However, laws do not conserve living animals, people do. The combined efforts of many people will be needed if these animals are to survive in the 21st century.

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CHAPTER 1

Food web dynamics and trophic level interactions of a multi-species community of freshwater unionids

INTRODUCTION

Laboratory studies on feeding behavior have shown that adult unionids can be classified as primary consumers, feeding primarily on algae, and to a lesser degree on bacteria and suspended detrital particles (e.g. Jørgensen, 1990; Prieur et al., 1990; McMahon, 1991; Vanderploeg et al., 1995; Silverman et al., 1997; Vander Zanden et al., 1997). However, gut content from a field study (e.g., Jiffry, 1984) found that unionids ingested not only primary producers such as algae, but also a substantial number of primary consumers such as cladocerans, copepods, and rotifers. The dietary information from these laboratory and field studies are limited in scope in that most test only one diet item at a time under atypical conditions, or rely on gut content or particle clearance rates to determine feeding preferences. While gut content data is frequently used to determine diets of other aquatic biota such as fish, it is of dubious use in unionids because of the problems differentiating between ingestion and assimilation. Determining the biological fate of ingested particles is difficult in unionids because they feed continuously, and sort food particles in the mantle cavity prior to ingestion and again in the stomach after ingestion. Furthermore, their digestive efficiency appears to be low, as much of the ingested material passes through the gut undamaged and alive (e.g., Miura and Yamashiro, 1990).

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Recently, food particle assimilation, as well as food web and trophic level relationships have been tracked in certain marine bivalves using stable isotope techniques (e.g., Incze et al., 1982; Fry, 1988). The purpose of our study was to apply these techniques to a multi-species community of freshwater unionids in their natural habitat. Our objectives were to determine the importance of specific food-web constituents of various species of unionids, and to determine the biochemical parameters of key dietary components.

The statistical and research hypotheses based on these objectives are as follows:

1. $H_0: \mu_1 = \mu_2$. All unionid species feed on the same food web components.
2. $H_0: \mu_1 = \mu_2$. The carbon and nitrogen stable isotope ratios of each unionid species are the same, indicating identical ingestion and assimilation of dietary components.
3. $H_0: \mu_1 = \mu_2$. The accumulation levels of various proteins, vitamins, and lipids between all unionid species is the same, indicating similar dietary requirements among species.
4. $H_0: \mu_1 = \mu_2$. All unionid species function as primary consumers, based on their stable nitrogen ratios.

Study Site

The unionids used in this survey were collected from two sites in southeast Michigan: the Huron River and Four Mile Lake (Figure 2). The Huron River is a regulated stream, consisting of a series of impoundments connected by free-flowing stretches of river. Two areas in the middle section of the river were selected for study. The first was located in the tailrace or dam outlet area of Portage Lake (Portage Lake tailrace). The second was a 4 km section downstream of the first location (Hudson Mills Park). The river in both areas is

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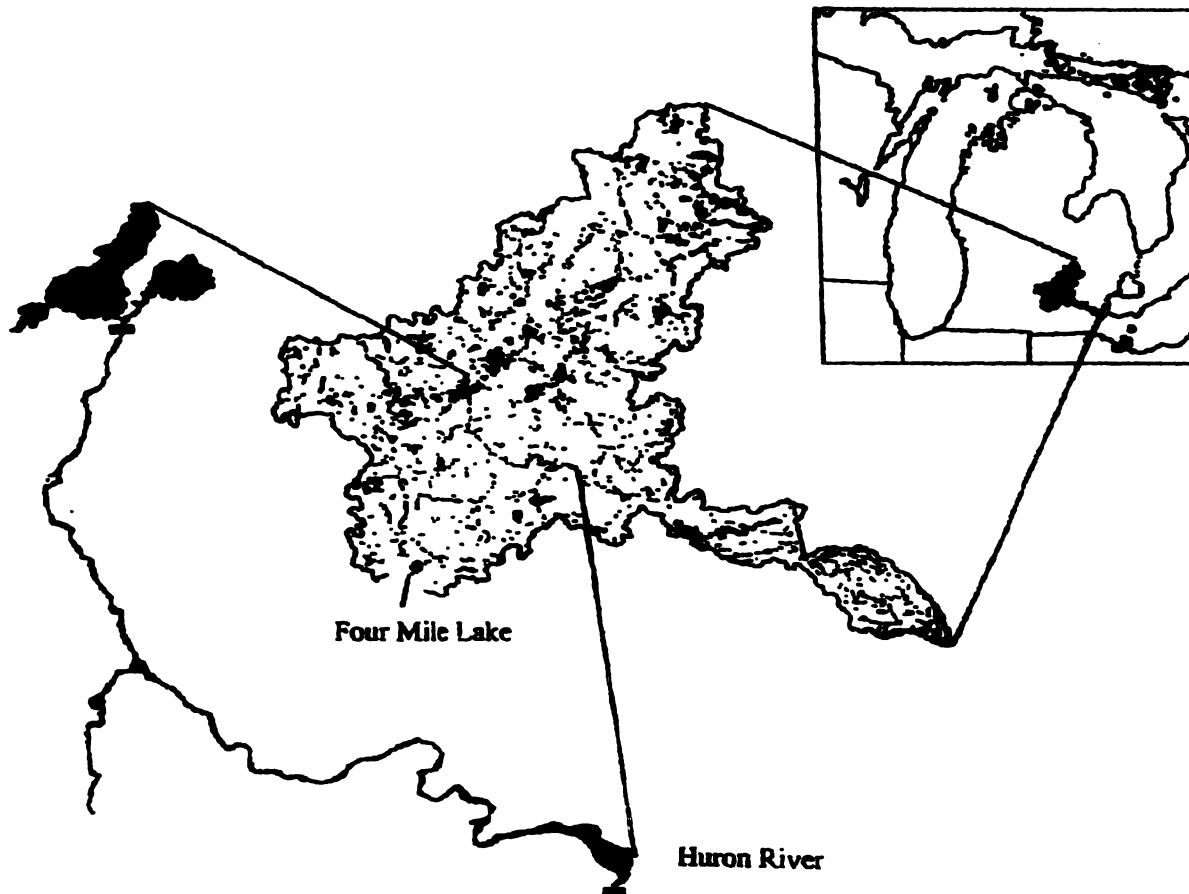
shallow (avg. 1 m deep), with a mean water velocity of 0.5m/sec and a riparian zone dominated by deciduous trees. These sites were selected because they contain the highest densities of unionids found in the river (up to 15/meter square) and have the highest species richness-- 16 unionid species (Figure 3). Our studies focused on seven of the most common species: *Cyclonais tuberculata*, *Elliptio dilatata*, *Lampsilis fasciola*, *Lampsilis ventricosa*, *L. siliquoidea*, *Ptychobranhus fasciolaris*, and *Pyganadon grandis*. The zebra mussel (*Dreissena polymorpha*), an exotic bivalve, has recently begun colonizing parts of the river. The Portage Lake tailrace unionids were upriver of the zebra mussels sampled, and the Hudson Mills Park unionids were downstream.

Four Mile Lake is a 25-hectare lake once used as a marl mining site. Except for the marl pit, the lake is shallow, ≤ 2 m deep, with no discernable flow, and the soft substrate is covered with macroalgae, *Chara* sp. Canopy cover is lacking, although some wetland vegetation grows along the edges of the lake. Only one unionid, *P. grandis*, regularly occurs in this lake. This lake is currently zebra mussel-free.

Figure 2. Locat



Figure 2. Location of the Huron River and Four Mile Lake in Southeast Michigan



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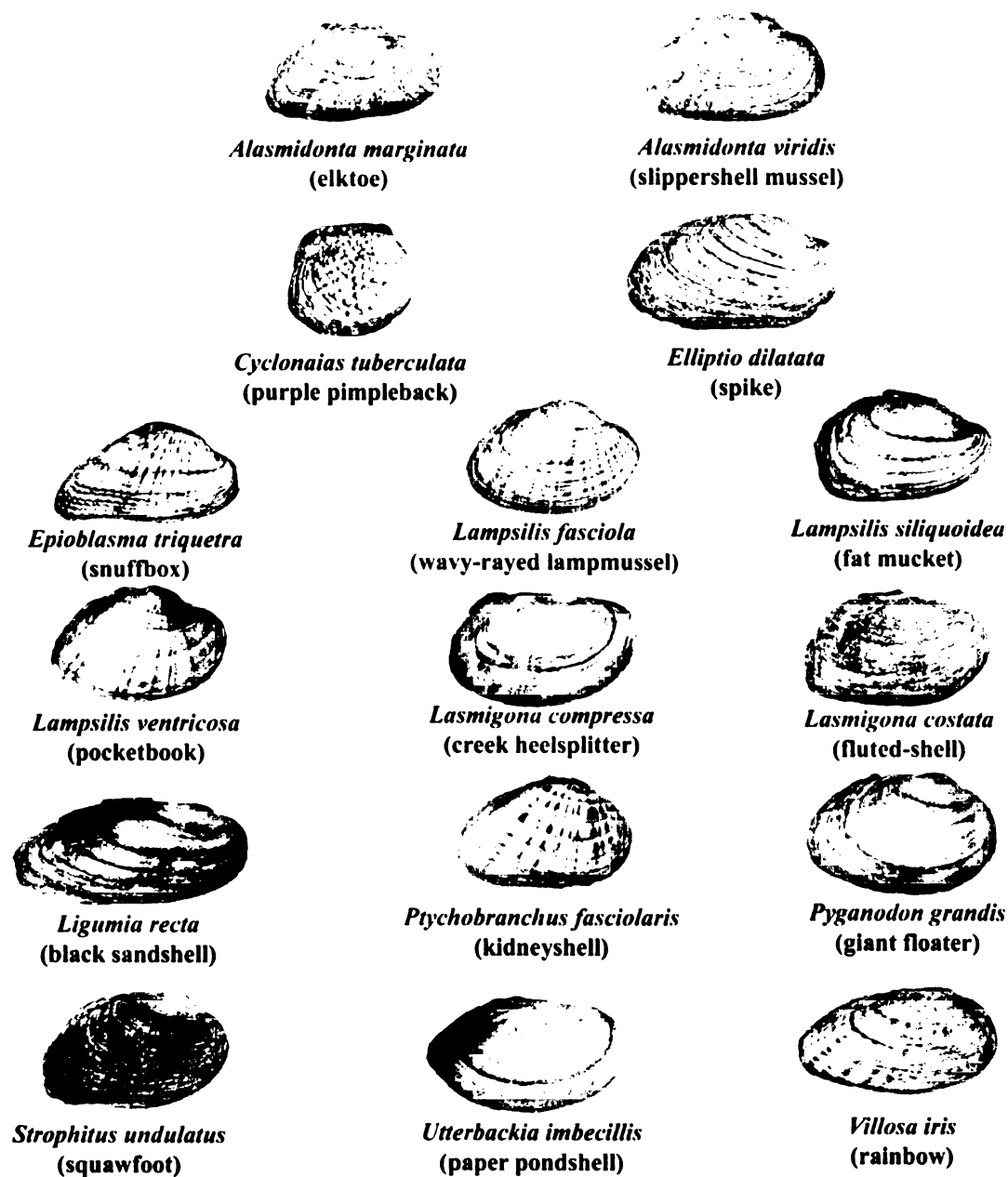
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Figure 3. Unionid species from the Huron River and Four Mile Lake, Michigan.



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METHODS

Food Web Components

A number of different sampling techniques were used to sample the food web in both the Huron River sites and in Four Mile Lake. Sampling for all components was conducted weekly from May to October 1996, in both the Huron River and Four Mile Lake. Sampling continued in the Huron River monthly from November 1996 to April 1997. The sampling station at Four Mile Lake was inaccessible, due to ice cover, from December to March. During each sampling period, three replicate samples of the following items were collected: benthos using an 8-cm core sampler, drift using a 23 μ mesh net (5- minute deployment in the river only), macrophytes (green leaves and stems of all species present), periphyton, plankton (23 μ mesh net used for 2-m oblique tows in Four Mile Lake), terrestrial vegetation (leaves, seeds, and twigs), and whole water samples (4 L/replicate). All samples were immediately placed on ice. In the laboratory, benthic samples, drift samples, and plankton samples were run through a series of Nitex sieves (1mm, 0.230 μ , 63 μ , 48 μ , and 28 μ mesh screen). Recognizable materials (diatoms, detritus, green algae, zebra mussels, zooplankton, etc.) were separated as needed under a microscope when clean samples of one particular item were required. A sample was considered clean if it contained < 5% other material. The terms < 28 μ , 28 μ , and 48 μ fine particulate organic matter (FPOM) or benthos refer to the sieved material obtained either from drift or plankton nets (FPOM) or benthic cores (benthos). This material was sorted further to type using percent composition methods described below. Periphyton and invertebrates were removed from the macrophytes in the laboratory and processed separately. Further processing of these samples depended on

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Whole water samples were used to provide data on total inorganic and organic particulate matter. Chlorophyll *a* levels were determined for both river and lake by filtering 4L/ replicate through 0.256 μ glass fiber filters and analyzed using spectrophotometric techniques described in APHA (1989). Results are presented in mg/m³. Total organic particulate matter (gm/L) was obtained by drying and ashing 100 mL of water and analyzing ash-free dry weights as described in APHA (1989).

FPOM samples (drift nets and plankton tows) were analyzed using percent composition. We used a modification of the areal standard, by taking a randomly selected 10 gm wet weight sample of the material, adding sufficient filtered well water to equal 25 mL, stirring, removing a 1mL aliquot, and placing this in a Sedgwick-Rafter cell divided into 5x5 mm grids. The estimated percentage of each type of material (detritus, zooplankton, green algae, etc.) was determined by estimating the percent coverage of each material in 10 randomly selected grid squares/1mL aliquot. Ten aliquots/sample were examined and the results averaged.

Taxonomic identifications of small invertebrate/phytoplankton species were made on each 1-mL aliquot in the Sedwick-Rafter cell after percent composition was determined. The entire 25-mL sample was examined for larger invertebrates, such as oligochaetes and chironomids, after the percent composition samples were removed.

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Gut and Mantle Cavity Contents

Five individuals of each of the seven species listed above were collected in March, August, and December 1996. These unionids were immediately placed on ice, and returned to the laboratory. At the laboratory, these animals were washed to remove external flora and then isolated in individual containers filled with filtered well water. Three individuals of each species remained in these containers over the next 24 hours, and all fecal matter (both digestive gland and intestinal) and pseudofeces were collected and contents examined. After 24 hours, these animals were wedged open and the mantle cavity flushed repeatedly with filtered well water. All flushed material was then examined and enumerated. Two of the five animals of each species were dissected after being thoroughly washed, and the contents of parts of the mantle cavity, digestive gland, and intestinal tract were removed and the percent composition and taxonomic diversity of the contents determined. Percent composition and taxonomic diversity of the contents removed from various parts of the unionid were processed as described above for the FPOM samples, except that all the material was used rather than being limited to 10 g. The unionid tissues were saved for stable isotope analyses and/or biochemical analyses.

Stable Isotope Analyses

Samples collected weekly and monthly using all the gear described above were processed individually and pooled randomly into 3 composite samples for each type of gear (e.g., FPOM) or food web component (e.g., chironomids) in order to reduce seasonal variability in isotopic composition. Unionids were individually processed, not pooled. Two individuals of each species were collected from both Huron River sites with the exception

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of *P. grandis* that only occurs at the tailrace site. Four individual *P. grandis* were collected from Four Mile Lake. Once samples were at the laboratory, each sample was cleaned thoroughly, with a brush if needed, and rinsed with deionized water. Each sample was flushed with 0.5 N HCL followed by several rinses with deionized water, dried at 58°C, ground into fine powder, and stored dry in a closed container until analyzed or pooled. The larger invertebrates saved for analysis were isolated in filtered well water for 3 days and then processed. Carbon (^{13}C) and nitrogen (^{15}N) stable isotopes were analyzed using isotope ratio mass spectrometry. Isotope ratios are expressed in delta (δ) format: $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ $= (R_{\text{sample}}/R_{\text{standard}})1000$, where $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ using PeeDee Belemnite standards for carbon and atmospheric air for nitrogen. Data are expressed as parts per thousand (‰). Analyses are accurate to $\pm 0.2\text{‰}$.

Biochemical Assays

Unionids and food web components used for biochemical assays were collected in the Huron River (*L. ventricosa*, *L. siliquioidea*, *P. fasciolaris*, and *P. grandis* from the tailrace site and *L. ventricosa*, *L. siliquioidea*, *P. fasciolaris* from Hudson Mills) and Four Mile Lake (*P. grandis*) in late August, 1996. The unionids collected were placed immediately on ice in the field and processed immediately on arrival at the laboratory. Animals were dissected, thoroughly flushed, and all soft tissues saved for analysis. Four animals of each species were used, and all tissue was pooled to form one composite sample for each species in order to obtain the required 400 g of wet material needed for analyses. Tissues were placed in sterilized glass jars and held at -40°C . Food-web-component samples were collections of drift net samples (28 μ mesh net), whole water samples filtered at the laboratory through a

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0.265 μ glass fiber filter, and plankton tows (Four Mile Lake) as described previously. Samples were pooled. All samples were tested for lipids (cholesterols, phytosterols, and total lipids), protein, and vitamins (A, C, B¹², D, E, biotin, choline, folacin, niacin, pantothenic acid, pyroxidine, riboflavin, and thiamin). Analyses were performed using standard high performance liquid chromatography (HPLC) techniques cited in AOAC International (1995).

Statistical Analyses

Statistical comparisons between samples were made using analysis of variance (ANOVA), and multiple *t*-tests. For all statistical tests, the outcome of each statistical test is considered significant at the $p < 0.05$ level. Analysis of variance procedures (completely randomized single-factor between-subjects ANOVA) were employed to evaluate differences in gut content ratios among plankton tows representing what food items were available ($n=3$) and unionid species ($n=5$ for each of three sampling dates for a total of 15, for the within-group variability) and between plankton tows and unionid species over time (seven species, $n=5$ for each of three sampling dates for a total of 45 animals, for the between-groups variability). Each food item, diatoms, detritus, rotifers, zooplankton, etc., was treated as an independent variable. Multiple *t*-tests (*t*-tests for two independent samples) were employed for simple comparisons of gut content ratios between unionid species and between plankton samples for each sampling date.

Stable isotope ratios were considered statistically significant if their respective range did not overlap. Carbon signatures were considered statistically significantly different ($p < 0.05$) if $\delta^{13}\text{C}$ values were $> 1.0\%$. Nitrogen signatures were considered statistically significantly

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different ($p < 0.05$) if $\delta^{15}\text{N}$ values were $>3\text{--}4\%$. The biochemical data are presented without statistical boundaries since only one pooled sample was analyzed. No within-species comparisons were possible since one unionid did not contain enough soft tissue to meet the weight requirements for analysis. Similarly, plankton tow samples were pooled in order to obtain enough material, by weight, to have analyzed.

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RESULTS

Food Resources

The Huron River and Four Mile Lake differed significantly in their chlorophyll *a* and total particulate organic matter (TPOM) (Figures 4 and 5). Chlorophyll *a* levels were significantly higher in the river than in the lake during the spring and early summer months, but had decreased below Four Mile Lake levels by late August. Huron River chlorophyll *a* peaked at 8.5 mg/m³ in July. Chlorophyll *a* in Four Mile Lake was at maximum levels of ≤ 4 mg/m³ by the end of August and remained there throughout the rest of the fall sampling until ice over.

Total organic particulate matter also differed seasonally, with the Huron River having significantly higher concentrations than the lake. As expected, peak particulates in the Huron River occurred in the spring during ice melt in the surrounding forests and during fall of the year when there were high leaf input. However, organic particulate concentrations were significantly higher in Four Mile Lake in the fall.

Gut and Mantle Cavity Components

Examination of the mantle cavity and digestive system of *Cyclonais tuberculata*, *Elliptio dilatata*, *Lampsilis fasciola*, *L. ventricosa*, *L. siliquoidea*, *Ptychobranchus fasciolaris*, and *Pyganadon grandis* (Huron River sites and Four Mile Lake) showed selective sorting of various food-web components from the water column. Representative examples are presented in Table 1. There was as much variability between individual replicates (n=5) for each species during each sampling date as there was between species.

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Figure 4. Chlorophyll a levels in the Huron River (■)(avg. both sampling sites) and Four Mile Lake (▲) in 1996.

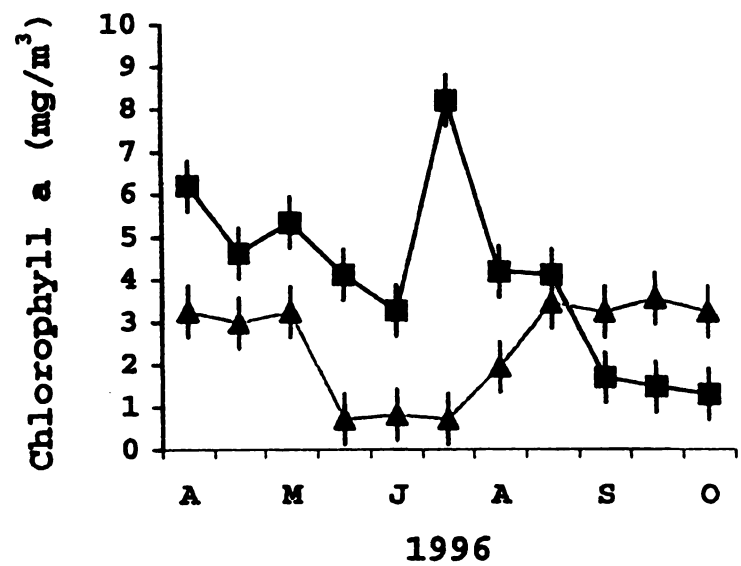


Figure 5. Total organic particulate matter in the Huron River(■) (avg. both sampling sites) and Four Mile Lake (▲) in 1996.

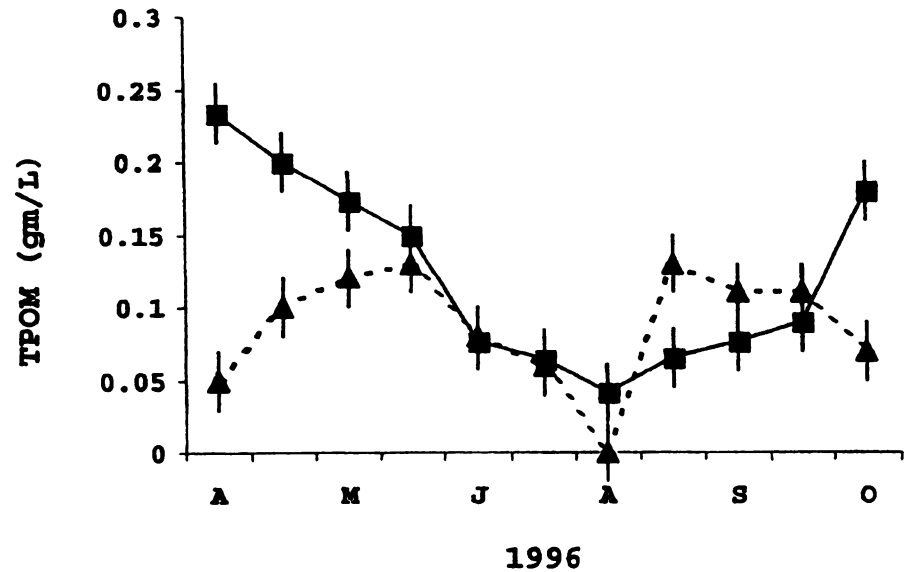


Table 1. Average % composition of material found in the mantle cavity (MC), digestive gland (DG),
Parasitoidia (*P. caudata*), *Lamprolaima* (*L. caudata*) and *Lamprolaima* (*L. caudata*)

Table 1. Average % composition of material found in the mantle cavity (MC), digestive gland (DG), intestinal tract (INT), of the unionids, *Pyganodon grandis* (*P. grand*) and *Lampsilis siliquoidea* (*L. siliq.*) as compared to the FPOM collected from Four Mile Lake and the Huron River, 1996. Unionid n=5/season, seston n= average of 3 plankton tows + 3 drift net samples (river only) + 3 whole water samples/season.

FOUR MILE LAKE												
			MARCH			JULY			NOVEMBER			
<i>P. grand</i>	MC	DG	INT	FPOM	MC	DG	INT	FPOM	MC	DG	INT	FPOM
Diatom	43.6 ±11.2	46.6 ±0.93	26.8 ±2.24	42 ±0.45	34.2 ±0.86	8.6 ±1.29	29.4 ±0.93	21.8 ±0.37	1.0 ±0	7.6 ±0.81	1.0 ±0	1.2 ±0.49
Green algae	42.4 ±16.7	48.6 ±3.16	55 ±9.84	42 ±0.63	30.2 ±10.6	89.6 ±1.17	32.6 ±0.51	42.8 ±0.73	38.0 ±1.41	89.9 ±1.16	47.8 ±1.39	50.8 ±3.38
Rotifer	4.8 ±0.86	0	1.6 ±0.45	1 ±0.5	6.0 ±0.71	0	2.2 ±0.49	3.0 ±2.51	8.0 ±0.71	1.0 ±0.32	4.2 ±1.07	2.4 ±0.75
Zooplank.	6.6 ±4.81	0	2.8 ±1.39	2.4 ±0.51	18.2 ±0.86	0	2.4 ±0.51	3.3 ±2.03	17.0 ±1.52	0.4 ±0.24	11.6 ±0.68	1.07 ±1.07
Detritus	2.6 ±0.68	1.8 ±0.49	14.6 ±0.98	13.4 ±1.12	9.4 ±0.93	0	33.4 ±8.75	11.6 ±0.51	36.0 ±1.84	2.0 ±1.50	34.6 ±12.0	34.2 ±0.8

Table 1 (cont'd). Average % composition of material found in the mantle cavity (MC), digestive gland (DG), intestinal tract (INT), of the unionids, *Pyganodon grandis* (*P. grand*) and *Lampsilis siliquoidea* (*L. siliq.*) as compared to the FPOM collected from Four Mile Lake and the Huron River, 1996. Unionid n=5/season, seston n= average of 3 plankton tows + 3 drift net samples (river only) + 3 whole water samples/season.

HURON R. <i>L. siliq.</i>	MARCH			JULY			NOVEMBER					
	MC	DG	INT	FPOM	MC	DG	INT	FPOM	MC	DG	INT	FPOM
Diatoms	13.5 ±5.90	90.0 ±2.50	19.9 ±11.50	15.9 ±2.60	5.0 ±1.0	2.0 ±0.05	0	0.6 ±0.45	2.0 ±0.1	10.0 ±2.25	0	1.8 ±0.37
Green algae	8.2 ±0.50	5.9 ±0.24	0	2.0 0	65.2 ±2.50	98.0 ±3.45	25.7 ±10.0	25.8 ±1.28	2.0 ±0.30	90.0 ±7.75	1.0 ±0.50	1.6 ±0.4
Rotifers	4.6 ±1.48	1.6 ±0.10	20.8 ±6.15	1.4 ±0.24	5.75 ±1.33	0	8.0 ±2.25	8.2 ±0.24	10.0 ±5.25	0	3.0 ±2.25	0.6 0±.24
Zooplank.	13.2 ±10.0	1.0 0	20.2 ±15.10	11.0 ±3.12	20.0 ±0.56	0	15.6 ±15.1	11.2 ±0.2	10.0 ±4.50	0	8.50 ±6.50	1.4 ±0.6
Detritus	60.5 ±9.40	1.5 ±0.24	54.5 ±15.00	70.0 ±5.50	17.9 ±1.00	0	50.7 ±19.5	54.2 ±1.59	76.0 ±10.5	0	75.0 ±10.7	94.2 ±5.00

Table 1 (cont'd). Average % composition of material found in the mantle cavity (MC), digestive gland (DG), intestinal tract (INT) of the unioidean *Dreissena polymorpha*.

Table 1 (cont'd). Average % composition of material found in the mantle cavity (MC), digestive gland (DG), intestinal tract (INT), of the unionids, *Pyganodon grandis* (*P. grand*) and *Lampsilis siliquoidea* (*L. siliq.*) as compared to the FPOM collected from Four Mile Lake and the Huron River, 1996. Unionid n=5/season, seston n= average of 3 plankton tows + 3 drift net samples (river only) + 3 whole water samples/season.

HURON R. <i>P. grand</i> Diatoms	MARCH				JULY				NOVEMBER			
	MC	DG	INT	FPOM	MC	DG	INT	FPOM	MC	DG	INT	FPOM
	14.6 ±3.51	97.0 ±0.63	13.0 ±10.22	15.9 ±2.60	0.8 ±0.2	4.0 ±3.63	0.2 ±0.2	0.6 ±0.45	1.8 ±0.2	8.6 ±1.29	1.0 ±0.50	1.8 ±0.37
Green algae	7.4 ±2.81	1.4 ±0.24	1.0 ±0.45	2.0 0	55.6 ±7.03	95.8 ±10.9	38.2 ±14.7	25.8 ±1.28	1.6 ±0.60	89.6 ±7.75	3.2 ±0.58	1.6 ±0.4
Rotifers	11.6 ±0.6	0.6 ±0.24	2.8 ±0.49	1.4 ±0.24	10.6 ±5.93	0	15.5 ±6.25	8.2 ±0.24	8.0 ±7.50	0	1.6 ±0.45	0.6 ±0.24
Zooplank.	14.2 ±9.01	0.6 ±0.24	19.6 ±12.13	11.0 ±3.12	21.0 ±6.45	0	21.6 ±8.43	11.2 ±0.2	17.0 ±1.52	0	2.8 ±1.4	1.4 ±0.6
Detritus	59.8 ±8.35	0.4 ±0.24	74.4 ±31.23	70.0 ±5.50	12.4 ±4.43	0	27.0 ±9.00	54.2 ±1.59	71.6 ±20.5	0	88.6 ±8.75	94.2 ±5.00

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The following table shows the ANOVA summary for averaged seasonal components of the food web items in the various unionid species in the Huron River and Four Mile Lake. In order to reject the null hypothesis of $H_0: \mu_1 = \mu_2$ that 'all the unionids feed on the same food web components' the F value must be greater than or equal to the F critical value.

ANOVA Summary seasonal food web components vs. all unionid species					
Source of Variation	SS	df	MS	F	F critical
Between species	513.7132	44	51.37132	0.064696	1.860439
Within a species	533.0031	4	94.04471		
Total	1046.7163	48			

Based on the fact that the F statistic obtained is smaller than F critical, the null hypothesis must be accepted, stating that based on gut data, all unionid species tested were feeding on the same food web component.

The variability in the ratio of dietary components found within an animal as compared to in the plankton was as high between species as within a species, indicating substantial individual differences in feeding rates and possibly preferences. The following tables summarize the two-tailed *t*-tests and show examples of the variability found.

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Two tailed *t*-test comparison of the statistical significance between the amount of diatoms found in the plankton in the Huron River versus the amount of diatoms found in all unionid species combined.

	% composition diatoms found in plankton in March, 1996, Huron River	% composition diatoms found in all unionids species in March, 1996, Huron River
Mean	22.22222	42
Variance	105.4444	1040.75
Observations	6	7
df	10	
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t Critical two-tail	2.228139	

In order to reject the null hypothesis of $H_0: \mu_1 = \mu_2$ that 'all the unionids feed on the same food web components' the *t*-statistic must be greater than or equal to the *t*-critical value. The *t*-statistic is smaller than the *t*-critical value, so according to gut content data, all the unionids are feeding upon the same food items and because of the difference between gut content versus plankton content, preferential selection of various food items was occurring.

The second example of a *t*-test summary shows the degree of variability between individual unionids of a single species, and the food items in the plankton. Variability among individuals of the same species was actually greater than the variability found among all unionid species combined. This high degree of variability emphasizes the individual differences in feeding periodicity and the inherent difficulties in using gut content for sampling in an animal that feeds continuously.

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	% composition diatoms found in plankton in March, 1996, Huron River	% composition diatoms found in individual <i>P. grandis</i> in March, 1996, Huron River
Mean	22.22222	27.55556
Variance	105.4444	1464.778
Observations	6	5
df	9	
t Stat	-0.40377	
t Critical	2.262159	
two-tail		

While this t-test also supports the hypothesis that all the unionids are feeding on the same items in the plankton, note the extremely high variance in the proportion of diatoms comprising the gut content in the individual *P. grandis*.

Although the variance in diet composition is high between species as well as within species, certain trends occurred in all species, all individuals and all locations. First, algae were more concentrated in the mantle cavity, digestive gland and intestinal tracts than in the surrounding waters. Secondly, algae dominated the content of the digestive gland, indicating a selection by the unionids for that item. Finally, over 50% of the invertebrates and algae found in the intestinal tracts and in fecal matter from the intestine were alive and undamaged. This was not the case with algae found in the digestive gland or in the digestive gland fecal matter. In both the Huron River and in Four Mile Lake, the dominant diatoms were *Asterionella* sp., *Ceratium* sp., *Cyclotella* sp., *Fragillaria* sp., *Navicula* sp., and *Nitzschia* sp., while *Ankistrodesmus* sp., *Anacyctis* sp., *Chlorella* sp., *Pediastrum* sp., *Scenedesmus* sp., and *Ulothrix* sp. were the most common non-filamentous blue-green and green algae. There was no preferential selection for specific algal species by any of the unionids- the genera of algae in the water column was basically identical to that found

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Stable Isotope

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inside the unionid. *Cladophora* sp. was the most dominant filamentous algae in the Huron River while *Chara* sp. dominated the substrate in Four Mile Lake- neither was found in the unionids.

The species composition and densities of the invertebrate community inside the mantle cavity of the unionids was diverse and numerous. Eighteen individual unionids (3 each of 6 species) from the Huron River, and 3 individuals of *P. grandis* from Four Mile Lake were processed. Examples of these data appear in Table 2. As with the gut content data, the variability in number and taxa found in each animal was significantly different in replicates of individuals within each species ($df=2$, $p<0.05$) as well as between species ($df=17$, $p<0.05$), so all 21 samples are significantly different from each other. The smaller invertebrate forms dominated this invertebrate community. Most of the larger insects, such as chironomids, were present as 1st and 2nd instar larvae. In both sites, the most common chironomid genera were *Cladotanytarsus*, *Dicrotendipes*, *Parakiefferiella*, *Polypedilum*, and *Tanytarsus*. The most common oligochaete genera were *Amphichaete*, *Chaetogaster*, *Enchytraeus*, *Lumbricus*, *Nais*, *Pristina*, and *Stylodrilus*. A number of lake zooplankton such as *Chaoborus*, *Holopedium*, and *Leptodora*, were collected only in Four Mile Lake.

Stable Isotopes

In the Huron River, the $\delta^{13}\text{C}$ ratios range from -21.6 ‰ (*Myriophyllum spicatum*) to -33.2 ‰ (*P. grandis* soft tissues) (Table 3; Figure 5). The most depleted elements were the unionid soft tissues, whose $\delta^{13}\text{C}$ ratios extend along a gradient, ranging from -31.9 ‰ for *C. tuberculata* to -33.2 ‰ for *P. grandis*. The unionids were more carbon-depleted than any other component of the food web, with the exception of fungus collected from the FPOM

Table 2. B
three ind:
Lake (4N)

Ciliates
8 taxa

Flatworms
2 taxa

Hydra

Gastrotrichs

Rotifers
4 taxa

Nematodes

Zebra mus
veligers

Glochidia*

Oligochaet
5 taxa

Tardigrad

* gloo
dying

Table 2. Examples of the invertebrate community colonizing the mantle cavity of three individual freshwater unionids from the Huron River (HR) and Four Mile Lake (4M) collected May, 1996. Actual number of invertebrates presented.

	<i>L. siliquoidea</i> -HR	<i>P. grandis</i> -HR	<i>P. grandis</i> -4M
Ciliates 8 taxa	34	82	51
Flatworms 2 taxa	12	51	12
Hydra	3	1	0
Gastrotrichs	31	6	0
Rotifers 4 taxa	1068	2117	989
Nematodes	42	61	83
Zebra mussel veligers	75	84	0
Glochidia*	885	12	0
Oligochaetes 5 taxa	89	114	51
Tardigrades	76	51	14

* glochidia of other species of unionids. All these glochidia were either dead or dying. All other animals were alive

Table 2 (continued)
cavity of
Four Mil
presented

Mites

2 taxa

Chironomidae
5 genera

Hemiptera

Trichoptera
2 genera

Cladocera
7 genera

Copepods
2 genera

Harpacticoid

Ostracods

TOTAL

Table 2 (cont'd). Examples of the invertebrate community colonizing the mantle cavity of three individual freshwater unionids from the Huron River (HR) and Four Mile Lake (4M) collected May, 1996. Actual number of invertebrates presented.

	<i>L. siliquioidea</i> -HR	<i>P. grandis</i> -HR	<i>P. grandis</i> -4M
Mites 2 taxa	8	28	48
Chironomid 5 genera	201	291	15
Hemiptera	1	2	0
Trichoptera 2 genera	2	1	0
Cladocerans 7 genera	45	65	101
Copepods 2 genera	2	3	60
Harpacti- coid	47	52	12
Ostracods	4	18	56
TOTAL	1147	3038	1492

(Figure 5). Based

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There was no sig

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Lake), *L. siliquo*

The $\delta^{15}\text{N}$ rat

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(Figure 5). Based on the degree of overlap between carbon signatures, the unionids were divided into three different groups: *P. grandis* was the most depleted (group 1), *C. tuberculata* was the most enriched (group 2), when compared to all other species (group 3). There was no significant difference in the carbon isotope ratio for species from the tailrace population above where zebra mussels occurred and that of the unionids from Hudson Mills below the zebra mussel-infested area. The shell $\delta^{13}\text{C}$ values were significantly more enriched than the soft tissue values for *P. grandis* (both in the Huron River and Four Mile Lake), *L. siliquoidea* (both river sites) and for *C. tuberculata*.

The $\delta^{15}\text{N}$ ratios for the Huron River food web ranged from a low of 1.2 ‰ (maple leaves) to a high of 12.0 ‰ for smallmouth bass. The $\delta^{15}\text{N}$ ratios for the unionids were, like the carbon values, extended along a gradient from 8.7 to 10.8 ‰ (Table 3; Figure 6). *Cyclonais tuberculata* has the lowest value (8.7), which was significantly different than that of *P. grandis* (10.8). The values for both these species were significantly different from the values of the other unionid species.

In Four Mile Lake, the *P. grandis* individuals also showed highly depleted $\delta^{13}\text{C}$ values, comparable to levels shown by *P. grandis* in the Huron River (Table 4; Figure 7), but food web components were more enriched and the ranges of values were greater, indicating less reliance on a microbial loop. The $\delta^{13}\text{C}$ values ranged from -18.1‰ for *Chara* to -32.2‰ for *P. grandis*. The $\delta^{15}\text{N}$ ratios were lower than found in the Huron River, ranging from a low of 4.1‰ (*Chara*) to a high of 8.5‰ for the minnow (*Notropus* sp.). *Pyganodon grandis* values averaged 7.8‰, significantly lower than the $\delta^{15}\text{N}$ levels for Huron River animals.

Table 3. Stability of
(S), and various
samples (n=3)
sample (n=4) o

<i>C. tuberculata</i>
<i>C. tuberculata</i>
<i>L. fasciola</i> T
<i>L. fasciola</i> S
<i>L. ventricosa</i>
<i>L. ventricosa</i>
<i>L. radiata</i> T
<i>L. radiata</i> S
<i>L. recta</i> T
<i>L. recta</i> S
<i>P. grandis</i> T
<i>P. grandis</i> S
<i>P. fasciolan</i>
<i>P. fasciolan</i>

Table 3. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) composition of unionid tissue (T), shell (S), and various food web components in the Huron River. Average of replicate pooled samples (n=3) for non-unionid material, $\pm\text{SE}$, collected seasonally in 1996. Average unionid sample (n=4) collected in December.

	Inorganic $\delta^{13}\text{C}$	Organic $\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>C. tuberculata</i> T		-31.9 $\pm$ 0.1	8.7 $\pm$ 0.2
<i>C. tuberculata</i> S	-10.3 $\pm$ 0.2	-31.4 $\pm$ 0.2	6.8 $\pm$ 0.4
<i>L. fasciola</i> T		-32.0 $\pm$ 0.1	9.8 $\pm$ 0.6
<i>L. fasciola</i> S	-12.9 $\pm$ 0.4	-31.7 $\pm$ 0.1	7.6 $\pm$ 0.2
<i>L. ventricosa</i> T		-32.5 $\pm$ 0.0	9.5 $\pm$ 0.2
<i>L. ventricosa</i> S	-13.0 $\pm$ 0.4	-32.6 $\pm$ 0.1	5.8 $\pm$ 0.3
<i>L. radiata</i> T		-33.0 $\pm$ 0.0	9.4 $\pm$ 0.1
<i>L. radiata</i> S	-13.4 $\pm$ 0.3	-31.4 $\pm$ 0.2	8.3 $\pm$ 0.4
<i>L. recta</i> T		-32.1 $\pm$ 0.1	9.8 $\pm$ 0.2
<i>L. recta</i> S	-12.6 $\pm$ 0.5	-31.9 $\pm$ 0.4	7.5 $\pm$ 0.1
<i>P. grandis</i> T		-33.2 $\pm$ 0.1	10.7 $\pm$ 0.2
<i>P. grandis</i> S	-13.0 $\pm$ 0.4	-32.7 $\pm$ 0.3	8.9 $\pm$ 0.1
<i>P. fasciolaris</i> T		-32.4 $\pm$ 0.2	9.4 $\pm$ 0.0
<i>P. fasciolaris</i> S	-11.6 $\pm$ 0.2	-32.4 $\pm$ 0.4	5.7 $\pm$ 0.1

Table 3. (cont.)
 shell (S), and var
 samples (n=3) fo
 sample (n=4) co

Diatoms
 Green algae
 Fungus
 Periphyton
 <23F FPOM
 28F FPOM
 48F FPOM
 Smallmouth ba
 (length= 10 cm
 Maple leaves
Myriophyllum
 River water
 (DOC)

Table 3. (cont.) Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) composition of unionid tissue (T), shell (S), and various food web components in the Huron River. Average of replicate pooled samples (n=3) for non-unionid material, $\pm\text{SE}$, collected seasonally in 1996. Average unionid sample (n=4) collected in December.

	Inorganic $\delta^{13}\text{C}$	Organic $\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Diatoms		-30.2 $\pm$ 0.1	3.0 $\pm$ 0.1
Green algae		-29.8 $\pm$ 0.3	3.1 $\pm$ 0.3
Fungus		-32.7 $\pm$ 0.0	5.4 $\pm$ 0.2
Periphyton		-31.0 $\pm$ 0.2	2.8 $\pm$ 0.2
<28F FPOM		-31.8 $\pm$ 0.1	6.4 $\pm$ 0.2
28F FPOM		-30.1 $\pm$ 0.1	6.3 $\pm$ 0.5
48F FPOM		-30.0 $\pm$ 0.3	6.2 $\pm$ 0.3
Smallmouth bass (length= 10 cm)		-31.0 $\pm$ 0.0	12.0 $\pm$ 0.0
Maple leaves		-27.1 $\pm$ 0.2	1.2 $\pm$ 0.1
<i>Myriophyllum</i>		-21.6 $\pm$ 0.0	4.6 $\pm$ 0.1
River water (DOC)		-19.0 $\pm$ 0.1	

Figure 6. ^{13}C ^{12}C
various biota coll

P. grandis T
P. grandis S

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L. siliq. S

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P. fascio. T
P. fascio. S

L. fasc T
L. fasc S

E. dilata T
E. dilata S

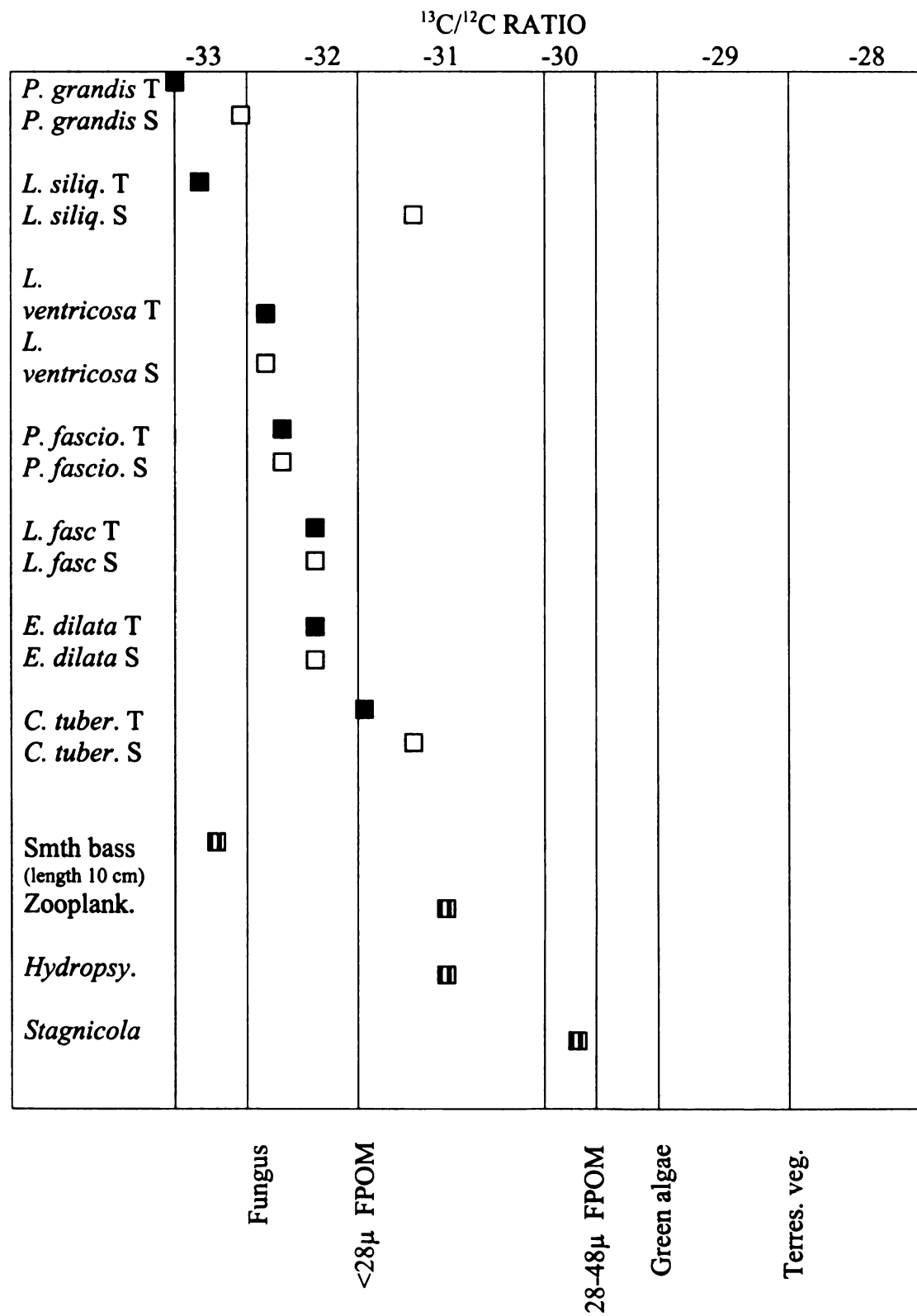
C. tuber. T
C. tuber. S

Smith bass
(length 10 cm)
Zooplank.

Hydropsy.

Stagnicola

Figure 6. $^{13}\text{C}/^{12}\text{C}$ stable isotope ratios found in unionid soft tissues (T), shell (S), and various biota collected from the Huron River (avg. both sampling sites) in 1996.



Biochemical Analysis

The unionid
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Biochemical Analyses

The unionid body soft tissues were relatively high in protein (50-60%) and averaged 10-13% in total lipids (Table 5). Glycogen was the most common remaining component in the tissues. The potential food items tested were by contrast, very low in protein and lipids, with the exception of zooplankton. The vitamin and sterol analyses found in the soft tissues are presented in Table 6. In general, all unionids bioaccumulated similar nutrients, such as cholesterol, phytosterols, the fat-soluble vitamins A and D, plus the water-soluble vitamins B₁₂, biotin, choline, folacin, niacin, pantothenic acid, pyroxidine and riboflavin. There was some indication of differences in vitamin uptake between species, but due to the limited sample size, few inferences can be drawn as to the variations in dietary requirements between species.

Table 4. Stable c
tissue (T1-T4), sh
Average of replic
Average unionid

<i>P. grandis</i> T ¹
<i>P. grandis</i> T ²
<i>P. grandis</i> T ³
<i>P. grandis</i> T ⁴
<i>P. grandis</i> S ¹
<i>P. grandis</i> S ²
<i>P. grandis</i> S ³
<i>P. grandis</i> S ⁴
Zooplankton
25μ benthos
45μ benthos
<25μ FPOM
Periphyton
Planktonic algae
48μ FPOM
25μ FPOM
<i>Chara</i>
<i>Hexagenia</i>
<i>Notropis</i>
Lake water (DOC)

Table 4. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) composition of four individual unionid tissue (T1-T4), shell (S1-S4), and various food web components in Four Mile Lake. Average of replicate pooled food web samples (n=6) $\pm$ SE, collected seasonally in 1996. Average unionid sample (n=4) collected in November.

	Inorganic $\delta^{13}\text{C}$	Organic $\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>P. grandis</i> T ¹		-32.3 $\pm$ 0.14	7.8 $\pm$ 0.11
<i>P. grandis</i> T ²		-32.2 $\pm$ 0.08	7.8 $\pm$ 0.11
<i>P. grandis</i> T ³		-32.0 $\pm$ 0.05	7.9 $\pm$ 0.13
<i>P. grandis</i> T ⁴		-32.1 $\pm$ 0.03	7.8 $\pm$ 0.09
<i>P. grandis</i> S ¹	-9.4 $\pm$ 0.01	-30.3 $\pm$ 0.02	7.3 $\pm$ 0.15
<i>P. grandis</i> S ²	-9.4 $\pm$ 0.02	-30.5 $\pm$ 0.03	7.2 $\pm$ 0.24
<i>P. grandis</i> S ³	-9.2 $\pm$ 0.00	-29.9 $\pm$ 0.05	7.4 $\pm$ 0.20
<i>P. grandis</i> S ⁴	-9.1 $\pm$ 0.00	-30.2 $\pm$ 0.01	6.9 $\pm$ 0.10
Zooplankton		-27.5 $\pm$ 0.10	8.5 $\pm$ 0.31
28 μ benthos		-27.1 $\pm$ 0.01	3.9 $\pm$ 0.28
48 μ benthos		-26.8 $\pm$ 0.22	3.0 $\pm$ 0.05
<28 μ FPOM		-26.6 $\pm$ 0.02	5.0 $\pm$ 0.03
Periphyton		24.0 $\pm$ 0.03	6.8 $\pm$ 0.20
Planktonic algae		-25.3 $\pm$ 0.11	5.6 $\pm$ 0.12
48 μ FPOM		-25.1 $\pm$ 0.08	4.6 $\pm$ 0.23
28 μ FPOM		-24.8 $\pm$ 0.05	4.7 $\pm$ 0.21
<i>Chara</i>	-9.2 $\pm$ 0.34	-30.1 $\pm$ 0.00	7.8 $\pm$ 0.14
<i>Hexagenia</i>		-28.3 $\pm$ 0.00	8.4 $\pm$ 0.30
<i>Notropus</i>		-25.0 $\pm$ 0.00	10.5 $\pm$ 0.13
Lake water (DOC)		-14.3 $\pm$ 0.00	

Figure 7. ^{13}C (‰)
T4), shell (S1-

<i>P.</i> <i>grandis</i> T1	
<i>P.</i> <i>grandis</i> S1	
<i>P.</i> <i>grandis</i> T2	
<i>P.</i> <i>grandis</i> S2	
<i>P.</i> <i>grandis</i> T3	
<i>P.</i> <i>grandis</i> S3	
<i>P.</i> <i>grandis</i> T4	
<i>P.</i> <i>grandis</i> S4	
Zooplan- kton	
Minnow	
Amphipo	
Hexagen	

	¹³ C/ ¹² C Ratio							
	-32	-31	-30	-29	-28	-27	-26	-25
<i>P. grandis</i> T1	■							
<i>P. grandis</i> S1			□					
<i>P. grandis</i> T2	■							
<i>P. grandis</i> S2				□				
<i>P. grandis</i> T3	■							
<i>P. grandis</i> S3				□				
<i>P. grandis</i> T4		■						
<i>P. grandis</i> S4			□					
Zooplankton						▣		
Minnow						▣		▣
Amphipo						▣		
Hexagen					▣			
	Chara			28μ benthos		<28μ FPOM		Diatoms/Green
								> 28μ FPOM

Table 5. Total pro
food web compon
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Table 5. Total protein content (% crude protein) and total lipids of various unionids and food web components from the Huron River and Four Mile Lake (unionid only) in August 1996. Samples based on dry weight.

	Protein	Lipids
<i>Cyclonais tuberculata</i>	69.5	10.0
<i>Elliptio dilatata</i>	63.5	12.7
<i>Lampsilis fasciola</i>	59.8	13.0
<i>L. ventricosa</i>	52.1	10.0
<i>L. siliquoidea</i>	60.5	12.0
<i>Pyganadon grandis</i> (Huron River)	55.2	12.5
<i>P. grandis</i> (Four Mile Lake)	52.0	10.5
<28 μ FPOM	6.7	≤ 1.0
28 μ FPOM	8.3	≤ 1.0
48 μ FPOM	16.3	≤ 1.0
Terrestrial Vegetation	4.1	≤ 1.0
Green algae	10.8	1.5
Zooplankton	40.0	3.0

Table 6. Lipid and
from the Huron B

	<i>L.</i> <i>ventri</i>
Chole- sterol ¹	34.5
Phyto- sterol ¹	25.4
Inosi- tol ¹	3.3
Vit A ²	135
beta Caro- tene ¹	0.0
Vit. D ³	84
Vit E ²	0.5
Vit C ²	1.
Vit B ₁₂ ³	35

Units of Meas
material.

Table 6. Lipid and vitamin content of select unionids and whole plankton/water samples from the Huron River and Four Mile lake. Pooled samples collected seasonally in 1996.

	<u>Huron River</u>					<u>Four Mile Lake</u>		
	<i>L.</i> <i>ventri</i>	<i>L.</i> <i>siliq</i>	<i>C.</i> <i>tuber.</i>	<i>P.</i> <i>fasc.</i>	<i>P.</i> <i>grand.</i>	Planktn	Planktn	<i>P.</i> <i>grand.</i>
Cholesterol ¹	34.5	52.1	43.2	46.0	48.1	3.0	2.7	51.8
Phytosterol ¹	25.4	26.2	20.0	21.7	11.0	9.0	9.0	18.8
Inositol ¹	3.3	2.0	3.0	2.5	10.0	2.0	2.0	5.9
Vit A ²	135	131	100	131	121	100	100	145
beta Carotene ¹	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.02
Vit. D ¹	84.2	105.0	79.7	96.0	62.3	25.0	25.0	41.9
Vit E ²	0.569	0.664	0.542	0.518	0.50	0.50	0.50	0.50
Vit C ²	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vit B ₁₂ ³	35.8	56.0	34.6	59.8	28.8	0.81	0.78	17.4

Units of Measurement: ¹= mg/100g dry material, ²= Iu/100g dry material, ³= F/100 g dry material.

Table 6 (cont'd).
 samples from the
 in 1996.

	<i>L.</i> <i>ven.</i>
Biotin ³	0.00
Choline ³	20.
Folia- cin ³	0.01
Niacin ³	0.8
Panto- thenic ³	0.2
Pyridox- -ine ³	0.0
Ribofla- vin ³	0.0
Thiamin ³	0.

Units of Meas
 material.

Table 6 (cont'd). Lipid and vitamin content of select unionids and whole plankton/water samples from the Huron River and Four Mile lake. Pooled samples collected seasonally in 1996.

	<u>Huron River</u>					<u>Four Mile Lake</u>		
	<i>L.</i> <i>ventri</i>	<i>L.</i> <i>siliq</i>	<i>C.</i> <i>tuber.</i>	<i>P.</i> <i>fasc.</i>	<i>P.</i> <i>grand.</i>	Planktn	Planktn	<i>P.</i> <i>grand.</i>
Biotin ³	0.0081	0.0078	0.0180	0.0175	0.0104	0.0005	0.0005	0.0162
Choline ³	20.0	20.0	20.0	20.5	20.3	4.0	4.0	17.1
Fola- cin ³	0.0152	0.0178	0.0101	0.0111	0.0117	0.006	0.010	0.011
Niacin ³	0.85	0.97	0.64	0.72	0.84	0.03	0.04	0.60
Panto- thenic ³	0.27	0.31	0.11	0.0.18	0.23	0.04	0.04	0.10
Pyridox -ine ³	0.014	0.015	0.011	0.012	0.010	0.007	0.01	0.04
Ribofla- vin ³	0.086	0.080	0.099	0.083	0.087	0.02	0.02	0.11
Thiamin ³	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Units of Measurement: ¹= mg/100g dry material, ²= Iu/100g dry material, ³= F/100 g dry material.

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DISCUSSION

Food Web Dynamics

Bacteria, not algae, were the main food resource of the unionids in the detrital-dominated Huron River as well as in the algal-dominated Four Mile Lake. While the gut content data shows that unionids were preferentially removing algae from the water column in both river and lake, algal carbons do not form a major portion of the soft tissue stores. This does not mean that algae are not critical dietary elements. Algal carbons were an important part of the shell matrix for some species and algal-derived vitamins and lipids were bioaccumulated by all unionids.

The bacteria that were incorporated into the unionids in the Huron River were associated with the $< 28\mu$ fine particulate organic matter (FPOM) and not the larger $> 28\mu$ FPOM. According to the carbon isotope ratios this FPOM fraction which consists of detritus, bacteria and various picoplankton, was the main food source of all Huron River unionids (Table 3; Figure 5). Given the premise that carbon isotopes undergo little fractionation during assimilation from the food source (Fry and Sherr, 1984), this portion of the FPOM was the only probable carbon source available to the bivalves. The carbon signatures for diatoms and green algae at -30.0‰ and -29.5‰ , respectively, are too enriched to be the dietary carbon source except possibly for *C. tuberculata* (-31.9‰).

One problem in tracking food resources was that the $< 28\mu$ FPOM has a more depleted $\delta^{13}\text{C}$ value than its individual components such as zooplankton and algae (Table 3; Figure 5). We believe that this discrepancy is the key to determining food web relationships of the unionids in this river and is the main support for our hypothesis that bacteria are the main

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food source. First, the highly depleted or negative $\delta^{13}\text{C}$ values are characteristic of bacterial or microbial decomposition of organic matter (Keough et al., 1998). Secondly, the biochemical data show that the unionids are assimilating bacterially derived compounds such as vitamin B¹² (Table 6). This vitamin is produced by certain starch-degrading bacteria, and is a key dietary requirement of cellulose-degrading bacteria. All the unionids tested bioaccumulated this vitamin as compared to levels found in other food web constituents. There is no evidence, however, to show whether the depleted carbons found in the unionid tissues are derived directly from bacteria or from bacterial exopolymers associated with the detritus. In all likelihood, the unionids are assimilating both individual cells as well as exopolymers. Certainly, on the basis of the stable isotope and biochemical data, we must assume that the detritus found in the mantle cavities and guts of all the unionid species is present due to positive selection, not accidental ingestion. Furthermore, these animals are probably feeding by “bacterial stripping”, by removing the bacteria and exopolymers associated with the detritus, as described for marine bivalves by Prieur et al. (1990). The role of bacterial exopolymers in unionid diets needs further investigation.

Bacteria also dominate the diet of the unionids in Four Mile Lake, even though more diatoms and green algae are present in the plankton as compared to the Huron River (Table 4; Figure 7). We had expected that the denser algal concentrations would lead to higher assimilation rates in the lake unionids resulting in a more enriched or positive $\delta^{13}\text{C}$ value. Peterson et al. (1986) has documented this kind of shifting in isotopic signatures due to the increasing importance of planktonic algae in marine bivalves as the population was sampled down an estuary and out to sea. However, the $\delta^{13}\text{C}$ values of *P. grandis* soft tissues in the

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lake are more depleted than the algal $\delta^{13}\text{C}$ values and are not statistically different from values found in the Huron River *P. grandis*. We are not sure why algal carbons are not being incorporated into the soft tissue in greater concentrations. Algae are more available and are readily ingested (Table 1). The question may be one of physiological fate of the algal carbons. Stable isotopes only track storage compounds not compounds utilized for energy production and excreted. Whatever the reason, the unionids at our test sites selected algae for ingestion, but with few exceptions were not retaining algal carbons as their dominant body carbon stores. The exceptions related to shell organic carbon values, where some species in the river, and the lake *P. grandis* contained significantly more enriched $\delta^{13}\text{C}$. We have inferred that these enriched carbons in the shell are algal derived based on the concentrated ingestion of algae into the gut. Our data does support the probability of an algal source, but does not exclude other sources such as the larger size fractions of FPOM.

Unlike the food web in the Huron River, we could not positively identify the source of the depleted carbon being utilized by the unionids in Four Mile Lake (Table 4; Figure 7). The carbon isotope ratios of all possible food web components are far too enriched to be providing the main source of soft tissue carbon stores of the unionid. The macroalgae, *Chara* sp. has the closest carbon isotope ratio to that of the unionids. This is interesting in that all the unionids collected in Four Mile Lake were found partially buried in dense mats of *Chara* sp. The unionids were obviously not feeding directly on this macroalgae, but there may have been feeding on some microbial community associated with it that we did not effectively sample. However, aquatic invertebrates commonly show either a more depleted $\delta^{13}\text{C}$ value or more enriched $\delta^{13}\text{C}$ value than any measured food supply due to problems in sampling microbial communities (e.g., Junger and Planas, 1993; France, 1995).

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Peterson and Fry
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The $\delta^{15}\text{N}$ values for the Huron River site support the same conclusions regarding diet as determined from the carbon isotope ratio (Table 4; Figure 6). The general assumption is that nitrogen isotope ratios will be enriched 3–4‰ between food item and consumer (see Peterson and Fry, 1987; Cabana and Rasmussen, 1994). All the unionid species are 3–4‰ enriched compared to the $< 28\mu$ FPOM, with the exception of *P. grandis* and *C. tuberculata*. The *P. grandis* $\delta^{15}\text{N}$ value is not that different, being only 4.7‰ higher than the $< 28\mu$ FPOM, but it is 7.7–7.8‰ higher than the values for green algae and diatoms. On the other hand, the $\delta^{15}\text{N}$ value of *C. tuberculata* is only 2.3‰ more enriched than the $< 28\mu$ FPOM and within 3–4‰ of the values for diatoms and green algae. Thus, *C. tuberculata* may be more herbivorous than the other species of unionids tested, or retain a higher fraction of algal carbons in its soft body stores. The nitrogen isotope data from Four Mile Lake did not provide any additional clarification of the lake food source of *P. grandis*.

The dietary relationship between unionids and the microbial loop is further complicated by the potential role of the invertebrate and microbial community inside the animal. We have shown that there is a dense invertebrate community colonizing the mantle cavity of these unionids, where some further processing of ingested material may occur before the unionid actually moves this material into its stomach. While no work has been done on freshwater unionids, the gills of marine bivalves are a main absorption site for free amino acids and other molecules (e.g. Stewart and Bamford, 1976). Although true endemic microflora are not present in unionids from Four Mile Lake and the Huron River (Chapter 2), unionids are believed to concentrate populations of transient bacteria inside their guts and mantle cavity. This concentration of transient unattached bacteria, such as *Vibrio*, is well

documented in marine bivalves (see review in Prieur et al., 1990), and preliminary studies indicate that it occurs in freshwater unionids as well (Starliper et al., 1997). This bacterial community in combination with the long gut retention time found in most unionids (3-5 day minimum, 2-week maximum Nichols, Unpubl. data), may account for some of the source of depleted carbon eventually assimilated by the unionids in 4 Mile Lake. It is an area needing further research.

Resource Partitioning

The stable isotope data shows that while all the unionid species are feeding from the < 28 μ FPOM, they are not feeding on, or assimilating nutrients from, the same FPOM components. The statistically different gradient in carbon ratios of the soft tissues and shell between *P. grandis*, *C. tuberculata*, and the other species reflects a mixing between depleted carbons from bacteria and other more enriched carbon sources. These enriched or more positive $\delta^{13}\text{C}$ could be derived from a number of sources including diatoms, green algae, zooplankton and the larger FPOM fractions. As mentioned earlier, the assumption is that algae are the primary source of these enriched carbons due to the preferential ingestion into the gut and digestive gland of both diatoms and green algae (Table 1). Identifying the source of the more negative or depleted $\delta^{13}\text{C}$ in *P. grandis* highlights one of the main limitations of our stable isotope data. We only separated clean samples of the more visible FPOM components such as algae and rotifers. We did not partition the smaller microautotrophs or herterotrophs, nor did we identify the bacterial community. Further identification of all FPOM components, perhaps using the techniques recently described by

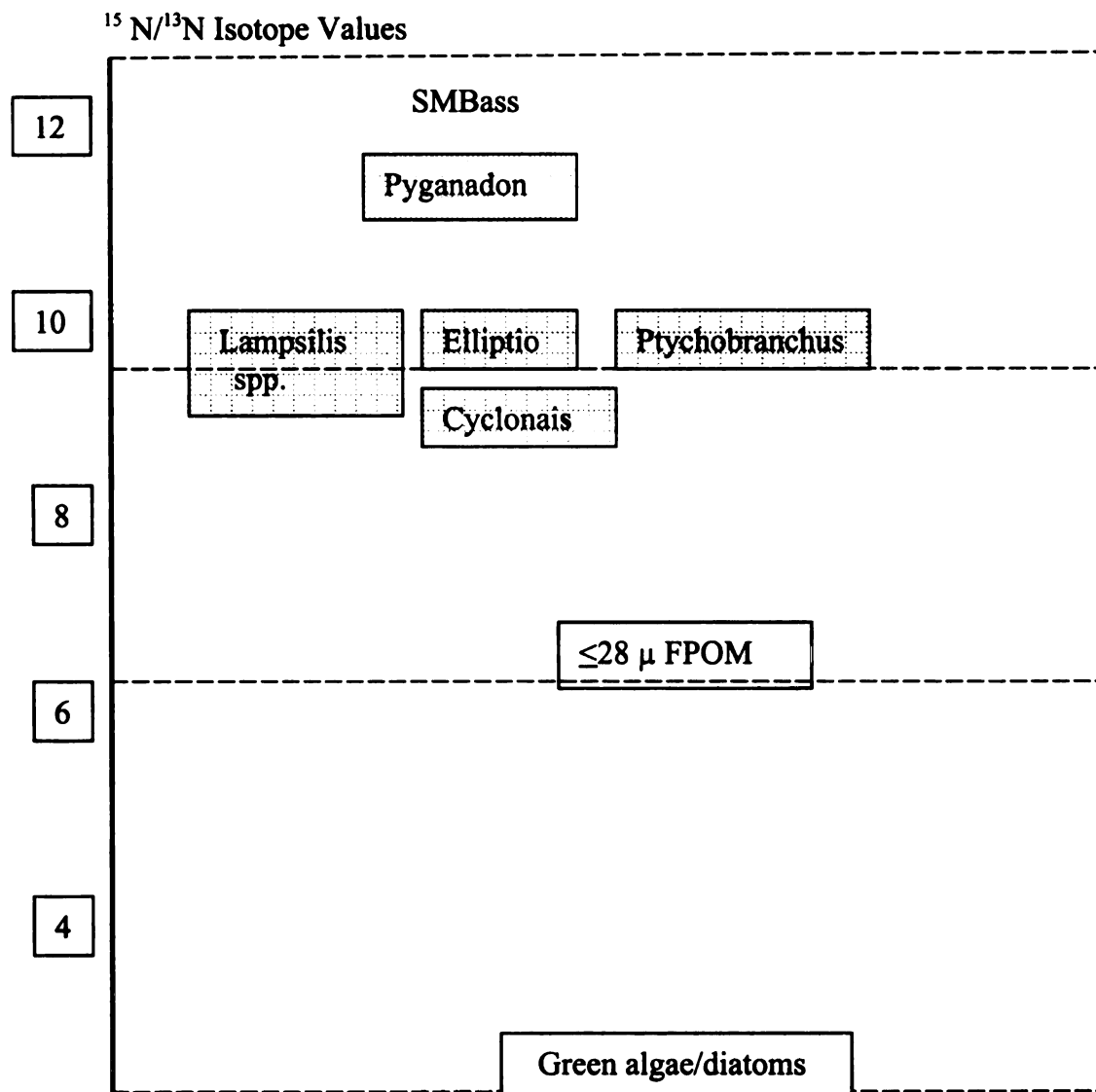
Hall and Meyer (1998) will be necessary to completely understand which components are actually being utilized and the physiological source of the various carbons stored by each unionid species.

Our stable isotope data was able to detect differences in the physiological fate of the more enriched carbons (algal? DOC in water?) within the various unionid species in the Huron River (Figure 5). Enriched carbons form a higher percentage of shell carbon than soft tissue carbons in *C. tuberculata*, *L. siliquioidea*, and *P. grandis*, but not in the other species tested, including the other *Lampsilis* spp. These differences in carbon incorporation may reflect differences in biochemical mechanisms, but may also be due to feeding behavior, capture potential or digestive capabilities.

Trophic Levels

The analyses of trophic levels in the Huron River based on $\delta^{15}\text{N}$ values of the unionids plus the known food supply as tracked by the carbon isotope ($< 28\mu$ FPOM) show that most of the species are primary consumers; they are one trophic level removed from their food supply (Figure 8). There is a problem with this designation that relates to the trophic classification of a mixed material such as the FPOM. Assigning trophic status assumes that primary consumers will have a nitrogen isotopic ratio about 3-4‰ higher than the primary autotrophs, secondary consumers would be 6-8‰ higher than the signal from the primary autotrophs and omnivores would fall in the middle (see Kling et al., 1992; Vander Zanden et al., 1997). If we assume that the FPOM is composed mainly of primary autotrophs, then the unionids fall within the primary consumer range, with the exception of *C. tuberculata* (+2.6‰) and *P. grandis* (+4.7‰).

Figure 8. Estimation of potential trophic relations between the various unionids and other biota from the Huron River based on $^{15}\text{N}/^{13}\text{N}$ stable isotope ratios. The dotted lines represent $\delta^{15}\text{N}$ values 3.5 ‰, or one trophic level apart. If the FPOM is primarily autotrophic, then most of the unionids would be primary consumers and *P. grandis* would be an omnivore.



However, the FPOM in this river may not be primarily composed of autotrophs. Kling et al. (1992) were able to assume their FPOM component was mostly composed of primary autotrophs due to its high algal content. However, the FPOM in our study is not principally algae, it is degraded detritus (Table 1). If we look at the $\delta^{15}\text{N}$ values of its basic components, the clean algal samples, and the terrestrial vegetation samples (Table 3), the unionids are so nitrogen-enriched as to be considered secondary consumers. If so, then *P. grandis* would be more on the level of a tertiary consumer, which seems a highly unrealistic and improbable concept. Assigning trophic level status to animals as closely linked to the microbial loop as unionids appear to be, will require more in-depth classification of the microbial communities in the FPOM at least into their autotrophic/heterotrophic ratios. There may also be numerous autotrophic picoplankton and microalgae present in the FPOM that were poorly represented by our clean algal samples. Turner and Roff (1993) have pointed out the difficulties in assigning trophic status to microbial loop communities because of the complexity of such systems and the present inability to differentiate the individual components. Certainly, the assumption cannot always be made that every unionid species are just primary consumers. Furthermore, although *P. grandis* is frequently used in laboratory studies on unionid feeding behavior because of its hardiness under laboratory conditions, the stable isotope data shows that it is atypical in its feeding habits compared to the other species tested.

The $\delta^{15}\text{N}$ values of the Huron River unionids are extremely high compared to nitrogen levels recently reported for unionids from the Canadian Shield lakes by Vander Zanden et al. (1997). Their survey on 36 lakes showed average $\delta^{15}\text{N}$ values for various unionid species of +4.2‰. Our values from the Huron River and Four Mile Lake are over twice what was

reported in their study. Such nitrogen enrichment is often characterized as indicating predation. While we do think our unionids are feeding on other primary consumers to some degree, on bacteria and microheterotrophic zooplankton, we hypothesize that the high nitrogen levels in the unionids in the Huron River relate more to anthropogenic influences than dietary habits. Wainright et al. (1996) found that organic pollution increased $\delta^{15}\text{N}$ values for the whole food web. The lake just above our sampling site is highly developed and has a history of septic system pollution into the lake. In comparison, the $\delta^{15}\text{N}$ values of *P. grandis* from a less developed site, Four Mile Lake, are 28% lower than that of the same species in the Huron River. (We did not assess trophic levels of *P. grandis* in Four Mile Lake since we could not identify the source of the bacteria).

Dietary Needs

The portion of the FPOM in the Huron River that the unionids were feeding on, the < 28 μ FPOM, could be considered low in lipids, protein and vitamins, but high in carbohydrates. This is similar to the nutrient composition determined for other FPOM from other sites (e.g., Peters et al., 1989). To survive on this protein-poor refractory material, the unionids would have to feed continually, which is a behavior noted in captive management of these animals. The bioaccumulation of various nutrients in both the Huron River and Four Mile Lake indicates that vitamins A, B₁₂, D, cholesterol and phytosterols are key dietary components for all the unionid species. Cholesterol is of special concern, since lipid studies on oysters show that this bivalve is incapable of forming cholesterol from various smaller chain precursors (Teshima, 1982). This lipid must be assimilated in its basic form

directly from the diet. Such nutrients should be incorporated into any captive diet formulations and in any food resource assessment of relocation sites.

CONCLUSIONS

Of the four statistical and research null hypotheses:

$H_0: \mu_1 = \mu_2$. All unionid species feed on the same food web components. On the basis of the gut content data, plus the stable isotope data, this null hypothesis could not be rejected. All unionid species tested were feeding on the same food web components, but to a differing degree of importance.

$H_0: \mu_1 = \mu_2$. The carbon and nitrogen stable isotope ratios of each unionid species are the same, indicating identical ingestion and assimilation of dietary components. Based on the stable isotope data, this null hypothesis was rejected with certain restrictions. One group of unionids, group 2, were feeding and assimilating basically the same material—they were in direct competition for the same food resource. *Pyganadon. grandis* and *C. tuberculata* were not in direct, overlapping competition with other unionid species, nor with each other for food resources.

$H_0: \mu_1 = \mu_2$. The accumulation levels of various proteins, vitamins, and lipids between all unionid species is the same, indicating similar dietary requirements among species.

The limited sample size prevented the acceptance or rejection of this hypothesis.

$H_0: \mu_1 = \mu_2$. All unionid species function as primary consumers, based on their stable nitrogen ratios. Based on the nitrogen isotope ratios, this hypothesis was rejected and the alternate hypothesis accepted. Not all unionids function as primary consumers.

Bacteria, not algae, were the main dietary item of all unionid species in both the detrital-based food webs in the river as well as the algal-based food web in the lake. Algae were preferentially ingested by the unionids, but algal carbons did not dominate the soft tissue stores of the unionids. Every unionid species bioaccumulated algal-derived compounds such as phytosterols, indicating the importance of algae in the diet. Other nutrients such as cholesterol, and vitamin B₁₂ also appear to be key dietary items. All the unionids could be considered primary consumers only if the assumption was made that the diet source, the $\leq 28\mu$ FPOM, was dominated by primary producers, which may not be the case in our river site. Further refinement of FPOM components will be necessary to complete our understanding of resource partitioning in this group of filter feeders. Until such time, a combination of sampling techniques, biochemical content, gut content, and carbon and nitrogen stable isotopes will be necessary in order to provide a comprehensive view of unionid dietary habits and requirements. Such information is critical for the future management of our declining unionid resources.

CHAPTER 2

The Role of Endosymbiotic Microflora in the Feeding Strategies and Nutritional Status of Freshwater Unionidae.

INTRODUCTION

As shown in Chapter One, all 16 species of unionids in the Huron River are feeding on the same food resource, the detrital-dominated $<28\mu$ FPOM (fine particulate organic matter). The stable isotope carbon and nitrogen data indicates that further resource partitioning of this FPOM fraction is occurring after ingestion, but not what mechanism is involved. In this chapter, we will examine the possibility that differences in endemic microbial flora play a major role in resource partitioning among the various unionid species.

In the Huron River, the FPOM is dominated by detritus that is composed primarily of cellulose derived from both terrestrial and aquatic vegetation, and secondarily of chitin derived from zooplankton carapaces and fungal cell walls. The role of these complex polysaccharides in unionid diets depends on whether they are directly digested or merely serve as substrates for actual dietary items such as bacteria and microciliates. Hypothetically, if unionids are using cellulose or chitin directly as a food resource, they must possess either a true primary cellulase and/or chitinase, capable of inducing primary bond breakage in these complex polysaccharides, or the unionids must have some type of symbiotic relationship with cellulolytic or chitinolytic microbes.

Endogenous enzymes capable of inducing primary bond breakage in cellulose and chitin are uncommon in aquatic invertebrates, and most detrital feeders such as black fly larvae

(Taylor et al., 1995) and crane fly larvae (e.g., Klug and Kotarski, 1980) access these enzymes through a symbiotic relationship with various microbes. Although endogenous cellulases have been reported for many marine bivalves, and a few freshwater species (e.g., Reid, 1968; Koopmans, 1970; Haag et al., 1993; Farris et al., 1994), true primary cellulases have been found in only a few marine species (Payne et al., 1972). Chitinase activity has not been reported for any freshwater or marine bivalves.

The few studies endosymbiotic bacteria in bivalves have focused on marine species and have yielded mixed results. Some marine species always contain endemic bacteria, particularly spirochaetes, attached to the epithelial layer of digestive tissue, and to the crystalline style (e.g. Bernard, 1970; Conway and Capuzzo, 1989; Prieur et al., 1990). Some marine bivalve species never contain such flora, while other marine bivalves demonstrate geographic variability, containing endemic microflora at some sites, but not at other sites (e.g. Bernard, 1970; Garland et al., 1982). Studies on freshwater bivalves are very few in number (e.g. Starliper et al., 1997) and have focused mainly on transient bacteria passing through the gut, which may, or may not be residential forms.

The presence or absence of endemic microflora in freshwater unionids is of interest as it relates to food web dynamics, and as a potential factor in the present poor relocation and aquaculture success of these animals. The continued extirpation of unionid populations throughout much of North America increases the need for developing successful techniques for relocating and captive management of these animals. Unfortunately, at this time, most unionid adults do not survive in captivity or in new locations for more than about three years (see Cope and Waller, 1995; Gatenby et al., 1999). While no work has been done on the

effect of sudden shifts in diet on gut flora of invertebrates, there is considerable information on the high mortality rates and/or poor dietary assimilation rates that can result from rapid diet changes in vertebrates that rely on endemic microflora as an aid in food processing. In order to test the hypothesis that unionids contained an endemic microflora that aids in the digestion of cellulose and chitin, we examined digestive tissues and contents from various unionid species on a seasonal basis from a number of different habitats.

The statistical and research hypotheses based on these objectives are as follows:

1. $H_0: \mu_1 = \mu_2$. Some, but not all, unionid species contain endosymbiotic microflora.
2. $H_0: \mu_1 = \mu_2$. Unionid species (*P. grandis* and *C. tuberculata*, Chapter 1) that showed significant differences in diets according to the stable isotope ratios, have distinctly different endosymbiotic microflora.

Study Sites and Unionids

The unionids used in this survey were collected from three sites in southeast Michigan: the Huron River, Four Mile Lake, and Vineyard Lake. The Huron River is a regulated stream, consisting of a series of impoundments connected by free-flowing stretches of river. Our study area focused on the middle section of the river, where the water is shallow (1m deep), with a water velocity of 0.5m/sec and heavy canopy cover dominated by deciduous trees. A total of 16 unionid species occur in this river. Four were selected for gut analysis: *Lampsilis ventricosa*, *L. siliquoidea*, *Ptychobranhus fasciolaris* and *Pyganodon grandis*. Four Mile Lake is a 25 hectare lake once used as a marl mining site. Except for the marl

pit, the lake is shallow, ≤ 2 m deep, with no current flow, and a soft substrate covered with *Chara*. Canopy cover is lacking, although some wetland vegetation edges the lake. Only one unionid, *P. grandis*, regularly occurs in this lake. Vineyard Lake is only 12 hectares in size, is 6.5 m deep, and is surrounded by subdivisions. Only one unionid, *Elliptio dilatata*, was observed in this lake.

METHODS

Microbial Gut Contents

Sampling for unionids occurred in March, August, and December, and 4 individuals of each species were collected. These unionids were removed from the field sites, placed in damp mesh bags in a cooler, and returned to the laboratory. At the laboratory, these animals were thoroughly washed to remove external flora and then dissected. Mantle cavity material and gut content were removed directly from those animals being prepared for dissection and scanning electron microscopy (SEM). The materials from each individual animal were placed in standard bacterial media, either cellulose, chitin, or cellobiose, and cultured aerobically or anaerobically at room temperature as described in Bryant and Burkey (substitute chitin for cellobiose, 1953), Bryant (1972), and Hungate (1950). Three replicate culture tubes were used for each animal and for each type of material. Culture tubes were examined for the presence of fermentation endproducts and media degradation after 24 hours, 48 hours, 72 hours, and 96 hours.

Examination for Attached Microbes

The mantle cavity tissue and gut tissue (including stomach, digestive gland, style, style sac, and intestine) were removed from the unionids as described above. The tissue from each animal was then immersed in 10% neutral buffered formalin (primary fixation). After the formalized tissue firmed, serial sections of approximately 1mm were taken using a scalpel. Digestive tissue was then isolated, trimmed, and washed three times for 20 minutes each in cacodylate-HCl buffer at a pH of 7.2. Secondary fixation involved immersion in 1%

cacodylate-HCl buffered osmium for one hour. Samples were then dehydrated in a graded alcohol series: 50% (15 min), 65% (15 min), 75% (15 min), 85% (15 min), 95% (15 min), 100% (3 changes 15 min each). Tissues were then critical point dried in CO₂ transition fluid mounted on 2.5 cm stubs, gold sputter coated, and examined under a scanning electron microscope.

Statistical comparisons between presence or absence of attached microbes within the unionids and growth on various types of media were made using analysis of variance (ANOVA), and two-sample *t*-tests. For all statistical tests, the outcome of each statistical test is considered significant at the $p < 0.05$ level. Analysis of variance procedures (completely randomized single-factor between-subjects ANOVA) were employed to evaluated differences in microbial response to various media ($n=72$) and in the presence of attached microbes ($n=24$). Multiple *t*-tests (*t*-tests for two independent samples) were employed for simple comparisons of gut content ratios between unionid species and between microbial/media samples for each sampling date.

RESULTS

Microbial Gut Contents

During periods of active feeding, all Huron River unionids contained cellulose-, chitin-, and cellobiose-degrading bacteria in both gut and mantle cavity contents (Table 7). The cellulose and chitin bacteria were different, but each were dominated by rod-shaped forms, were fermenters, and facultative anaerobes (Figure 9a and b). The cellobiose-degrading bacteria were dominated by cocci, were also fermenters, and facultative anaerobes. In

December, when these river animals were not feeding, cultures of material removed from the unionids lacked any sign of cellulose- or chitin degrading bacteria, but cellobiose-degrading bacteria were present.

Results from the lake unionids differed. Neither *P. grandis* from 4 Mile Lake nor from *E. dilatata* from Vineyard Lake contained cellulose- or chitin-degrading bacteria regardless of feeding activity (Table 7). However, like the Huron River animals, these lake unionids consistently showed cellobiose activity with a bacterial community dominated by cocci that were fermenters and facultative anaerobes.

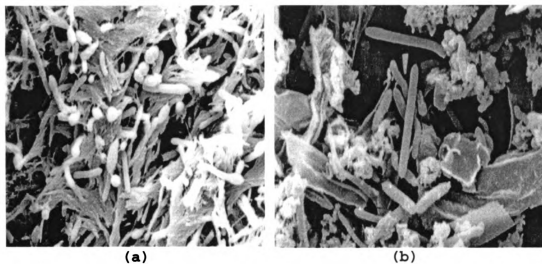
Examination for Attached Microbes

Examination of tissues using the scanning electron microscope showed no attachment to digestive or mantle tissues of the rod-shaped cellulose- or chitin-degrading bacteria during any season, in any unionid, from any sampling site. No attached bacteria of any kind could be found on the epithelial surface of various mantle and digestive organs such as the stomach, ciliary tufts, crystalline style and style sac, digestive gland, or the upper intestinal.

Table 7. Response of mantle cavity and intestinal tract residue to various bacteriological media. Response code: + means media degradation after 24 hours of culture; 0 indicates no response.

		Cellulose	Chitin	Cellobiose	Attached Spores
March and Aug.	<i>Lampsilis ventricosa</i>	mantle +	mantle +	mantle +	present
		gut +	gut +	gut +	
	<i>Lampsilis siliquouidea</i>	mantle +	mantle +	mantle +	present
		gut +	gut +	gut +	
	<i>Elliptio dilatata</i>	mantle +	mantle +	mantle +	present
		gut +	gut +	gut +	
	<i>Pyganadon grandis</i> Huron River	mantle +	mantle +	mantle +	present
		gut +	gut +	gut +	
	<i>Pyganadon grandis</i> Four Mile Lake	mantle 0	mantle 0	mantle +	present
		gut 0	gut 0	gut +	
Dec	<i>Lampsilis ventricosa</i>	mantle 0	mantle 0	mantle +	present
		gut 0	gut 0	gut +	
	<i>Lampsilis siliquouidea</i>	mantle 0	mantle 0	mantle +	present
		gut 0	gut 0	gut +	
	<i>Elliptio dilatata</i>	mantle 0	mantle 0	mantle +	present
		gut 0	gut 0	gut +	
	<i>Pyganadon grandis</i> Huron River	mantle 0	mantle 0	mantle +	present
		gut 0	gut 0	gut +	
	<i>Pyganadon grandis</i> Four Mile Lake	mantle 0	mantle 0	mantle +	present
		gut 0	gut 0	gut +	

Figure 9. Scanning electron micrograph of the bacterial community cultured from the mantle cavity and gut content of freshwater Unionidae. **(a)** shows the bacterial community that developed in cellulose media, while **(b)** shows the community that developed in chitin.



tract of any species (Figure 10a-d). All epithelial layers of these tissues were covered in March and August with a heavy layer of mucus. Food particles, including bacteria, were often seen trapped in this mucus layer (10e and f), but no attached bacteria were associated with this mucus layer

Every unionid examined, from all sites, contained attached spores in their intestinal tracts. These spores were located below the mucus layer and between the enterocytes comprising the intestinal wall (Figure 11). The associated spores were large, about 1 micron in diameter (Figure 12a-f), and were attached through a stalk (Figure 12d and f). Attempts to rear these spores in isolation, for further elucidation, failed. The mantle tissues of most of the unionids examined did contain an assortment of attached bacteria and fungus (Figure 13), although attachment patterns seemed to be random. The labial palps and the gills lacked any associated microorganisms.

Statistical analyses were not performed as originally planned due to the homogeneity of response. For example, all of the unionids contained attached spores in their intestinal tracts, none of the attempts to culture cellulose-degrading microbes from Four Mile Lake were successful, etc.

Figure 10. Scanning electron micrograph of the unionid digestive tissue examined for the presence or absence of endosymbionts. (a) Crosssection of the stomach, intestine, and digestive gland; (b) digestive gland; (c) surface of crystalline style showing mucous sheet and food bolus; (d) ciliary tuft found in the intestinal tract; (e) diatom buried in mucous sheet on the surface of the intestinal tract; (f) mucous sheet and food material (diatoms and transient bacteria) on the surface of the intestinal tract.

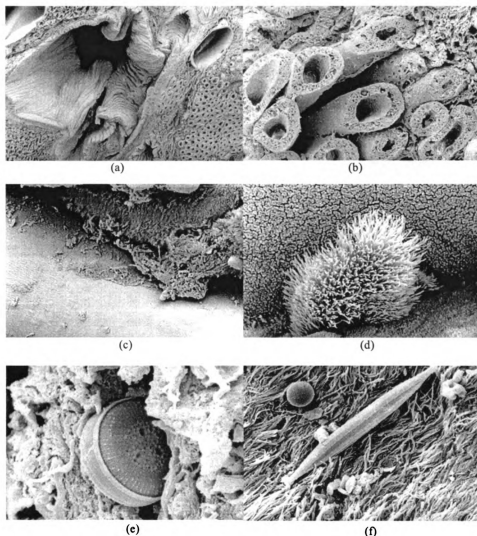


Figure 11. Scanning electron micrograph of unionid intestinal tract showing ciliated enterocytes and the loose junctions between the cells. The attached spores are found between these enterocytes.

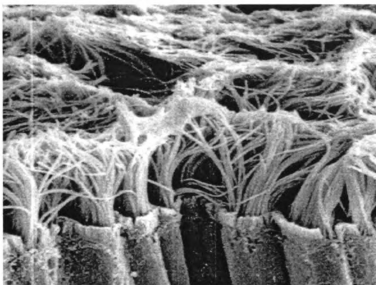


Figure 12. Scanning electron micrographs of the spores found in the intestinal tract of various species of freshwater unionids collected from several sites in southeast Michigan.

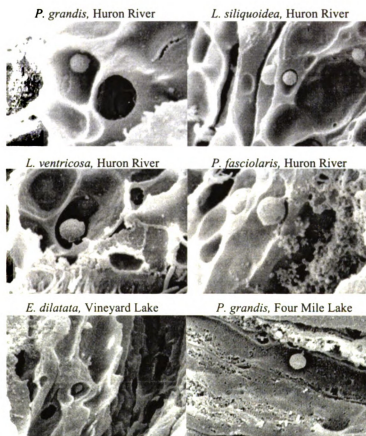
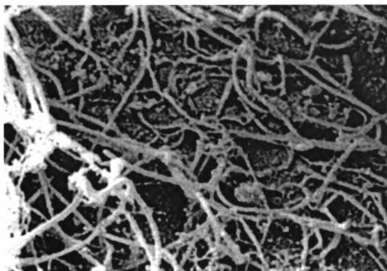


Figure 13. Scanning electron micrograph of the fungus attached to the mantle tissue of a single *Lampsilis siliquoidea*



DISCUSSION

None of the unionids examined contain a true endemic microflora, based on the criteria used for vertebrate fauna (see criteria discussion in Yokoyama and Johnson, 1993). While technically the spores or fruiting bodies found attached to tissues inside all the unionids could be considered endemic microflora based such criteria, there are problems associated with this designation. As with true endemic microflora, these attached spores had a widespread distribution, being found in all unionid species from every location (e.g., the Huron River, Four Mile Lake, and Vineyard Lake); the spores were not seasonally limited as they were found even in the winter when the unionids were not feeding; and the spores were consistently found attached to the same tissues, the intestinal walls. However, two key criteria for endemic fauna have not been met. The first problem relates to densities. In vertebrate and even other aquatic invertebrates, endemic fauna associated with the digestive process are present in numbers in excess of $>10^{10}/g$ and cover the surfaces of associated gut tissues (e.g. Yokoyama and Johnson, 1993; Taylor, et al. 1995). In contrast, the spores found associated with unionid mantle and intestines (see Figures 12 and 13) though consistently present, were so low in densities that their importance as an active partner in the digestion of food resources is severely limited. The second problem is that the spores are fruiting bodies, and the microbe producing them was never identified or found attached inside the unionids.

In appearance and size, these spores resemble the fruiting bodies produced by fungus. It is possible they are produced by a mat-forming bacterium. If so, finding such microbial forms in unionids will be difficult because of the large amount of mucus present. Unionids produce vast quantities of mucus as part of their food-handling process. This mucus forms

thick layers in along all epithelial tissues both in the gut and in the mantle cavity. Mucus strands hide the surface of the epithelial layers and in appearance under SEM, resemble the shape of mat-forming bacteria or fungus. While heavy mucus production adds difficulties to endemic microbe identification, if fungus or a mat-forming bacterium was a common, endemic microbe in unionids it would have been found in its vegetative form. This is particularly true with the winter samples when mucus production is lower. Fungal hyphae attached to unionid mantle tissue were identified in only one animal (Figure 13) and it was not producing spores at the time. Since fungus was extremely common in the detrital particulate matter drifting in the river and since detritus forms such a substantial portion of the diet of all the unionids (see Chapter 1), the spores associated with the unionid, may simply represent remnants of transient fauna.

None of the unionids contained resident microbes capable of digesting cellulose or chitin. The cellulose- and chitin-digesting bacteria, all rod-shaped microbes, which were cultured from intestinal and mantle cavity material, have all the characteristics of truly transient fauna. These microbes were never associated with or attached to unionid tissues, were seasonal in their presence (absent when the unionids were not feeding) and habitat limited to Huron River unionids (Table 7). It is surprising that no cellulose or chitin microbes could be cultured from the Four Mile Lake animals. This lack must reflect the feeding habits of the unionids in the lake and site-specific peculiarities in the microbial loop.

Food Web Dynamics

Since all the species of unionids tested in the Huron River contained the same type of attached spores, and no other attached microbes, endemic microbial fauna are not a

mechanism by which multi-species communities of unionids partition shared food resources. Even species such as *P. grandis* and *C. tuberculata* that showed substantial differences in food web dynamics and trophic level status (Chapter 1) do not contain different symbiotic microbes. Similarly, the results for the presence of microbes capable of digesting cellulose or chitin indicate unionids are a reflection of what is available in their environment, not by species, in regards to microbes capable of digesting these complex polysaccharides.

One problem in determining the roles of transient and endemic microbial fauna in bivalve nutrition and resource partitioning is that the model used, which was developed for vertebrates, is not readily applicable to animals as closely associated with the microbial loop as are unionids. Bivalves in general, and unionids in particular, unlike vertebrates and some invertebrates such as termites, apply both selective predation and environmental enhancement to microbial communities ingested during feeding. Unionids feed on certain microbes, as shown in Chapter 1 and in work by Silverman et al. (1995, 1997). The population densities of other microbes are enhanced in the intestinal tract as compared to surrounding waters (Starlipper et al., 1997). As with marine bivalves, proteolytic bacteria such as *Vibrio* spp, dominate this enhanced community. The continuous feeding behavior of the unionids along with the slow gut retention time provides a continual replacement of microbial fauna in the gut. These are not true endemics as defined in vertebrates and in some invertebrates, as this microbial fauna is lost as soon as feeding ceases; the microbes are shed during depuration within 3-4 days. Further research is needed in order to determine if this altered transient community differs between unionid species and provides selective advantages to various species in competition for food.

CONCLUSIONS

The data shows that the statistical and research hypotheses should both be rejected and the alternative hypothesis accepted:

$H_0: \mu_1 = \mu_2$. Some, but not all, unionid species contain endosymbiotic microflora.

Since only spores were found, not the attached vegetative structures, no unionids contain true endemic fauna.

$H_0: \mu_1 = \mu_2$. Unionid species (*P. grandis* and *C. tuberculata*, Chapter 1) that showed significant differences in diets according to the stable isotope ratios, have distinctly different endosymbiotic microflora.

The null hypothesis was rejected as there was no difference in endosymbiotic microflora between unionid species.

All the unionid species tested, from all locations lack endemic microbial fauna with the exception of a few attached spores. These spores are limited in number that and the vegetative form was never found, so this microbe does not play a role in partitioning of shared food resources between unionid species. The lack of endemic cellulolytic and chitinolytic microbes should not influence relocation success of unionids as these microbes can be found in many environments. This lack would influence captive maintenance success if dry diets based on these complex polysaccharides were fed without prior microbial predigestion.

CHAPTER 3

Benthic Feeding in Adult Freshwater Bivalves.

INTRODUCTION

Freshwater bivalves can have a major impact on the bioenergetics of aquatic ecosystems. Bivalves are the largest group of freshwater invertebrate species known and they often dominate the benthic community biomass, at times reaching population densities $>700,000/\text{m}^2$ (*Dreissena polymorpha*, Kovalak et al., 1993). Such high population densities remove a large percentage of food resources from the water column and substantially alter the food web dynamics for the entire ecosystem. For example, Madenjian (1995) estimates that zebra mussel populations in Lake Erie remove up to 36% of the lake's yearly primary productivity. These energy budgets assume that adult bivalves rely solely on planktonic food sources obtained through filter feeding and that food materials not suspended in the water column are not available as food sources.

Benthic food sources comprise a major portion of the diet of newly transformed juveniles (non-veligers) of all freshwater bivalves through the use of pedal (foot) feeding until the gills and siphons completely develop (McMahon, 1991; Gatenby et al., 1994). This ability to foot feed is reportedly lost within the first year of life for most species with the exception of adult Pisidiidae/Sphaeriidae (Hornbach et al., 1984; Lopez and Holopainen, 1987; Way, 1989), and in adult *Corbicula fluminea* (Way et al., 1990; McMahon, 1991; Reid et al., 1992). All these species known to access benthic foods even as adults tend to be very small and their siphons are frequently in direct contact with the substrate. Adult unionids and the epibenthic dreissenids have been classified as suspension feeders, relying solely on

the inhalant siphon to gather suspended organic particles and as such benthic food resources would, theoretically, be of minimal importance (Morton, 1973; McMahon, 1991).

Recent studies on unionids and dreissenids have raised questions concerning the possibility that benthic food resources may comprise a substantial portion of the diets of adult freshwater bivalves in some locations. In Chapter 1 and in Raikow (1999) the assimilation of benthic foods in separate unionid communities from two locations has been documented. There are also questions regarding the food resources of the deep-water *Dreissena bugensis* that are epifaunal, buried in the substrate in the deep waters of Lakes Erie and Ontario. In this Chapter, we will test the possibility that many species of freshwater bivalves retain the ability to access benthic food sources even as adults and are not solely limited to feeding on organic particles suspended in the water column.

The statistical and research hypotheses based on these objectives are as follows:

1. $H_0: \mu_1 = \mu_2$. Water intake into the bivalve is limited to the inhalant siphon.
2. $H_0: \mu_1 = \mu_2$. Water outflow into the unionid is bi-directional through the inhalant siphon and unidirectional through the exhalant siphon.
3. $H_0: \mu_1 = \mu_2$. Water intake and outflow patterns differ between *C. fluminea*, *D. polymorpha*, and all the unionid species.

METHODS

Test Animals

We tested both non-siphon and siphon feeding behavior in 19 species of freshwater bivalves (Table 8). The inhalant siphon types among the animals ranged from well developed long tubes of *Corbicula fluminea* (Asian clam) and *Dreissena bugensis* (quagga mussels) to the non-tubular siphon of the unionid, *Cyclonaias tuberculata*. Animals used in the experiments were chosen regardless of sex or reproductive status. All bivalves were held in well water for 10 days prior to testing under conditions previously described for zebra mussels, *Dreissena polymorpha* (Nichols, 1993), except that no food was provided. The test water was filtered well-water, pH 7.5, dissolved calcium 175 mg/L, photoperiod 12 light/12 dark, and temperature 20°C. The food used in the tests was a dried green *Chlorella* or live *Chlamydomonas reinhardtii*.

Testing procedures

Three types of tests were used to detect non-siphon, siphon, and pedal feeding behaviors: (1) exposing the anterior end of the animal to a planktonic food supply, while at the same time, completely isolating the posterior inhalant and exhalant siphons from the food supply, (2) exposing the entire animal to a benthic mat of nonmotile algae, and (3) providing the entire animal with a planktonic food supply, but inhibiting extension and use of the siphons.

Table 8. Mantle area where food enters and pseudofeces leave the mantle cavity of 19 species of freshwater bivalves used in feeding behavior studies. Ant = anterior portion of shell/mantle cavity, Pos = posterior inhalant siphon, Midl = medial part of shell/mantle cavity anterior to the inhalant siphon, Bys = byssal opening in shell/mantle. Shell (cm)= avg. ventral shell length.

	Shell (cm)	Food Enters Thru Shell	High Viscosity Pseudofeces Ejected thru:	Low Viscosity Pseudofeces Ejected Thru:
<i>Alismodonta marginata</i>	11	Ant/Pos	Midl/Pos	Ant/Pos
<i>Amblema plicata</i>	13	Ant/Pos	Midl/Pos	Ant/Pos
<i>Cyclonaias tuberculata</i>	8	Ant/Pos	Midl/Pos	Ant/Pos
<i>Elliptio complanata</i>	10.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Elliptio dilatata</i>	8	Ant/Pos	Midl/Pos	Ant/Pos
<i>Lampsilis fasciola</i>	5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Lampsilis radiata</i>	12.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Lampsilis ventricosa</i>	14	Ant/Pos	Midl/Pos	Ant/Pos
<i>Leptodea fragilis</i>	9	Ant/Pos	Midl/Pos	Ant/Pos
<i>Ligumia subrostrata</i>	7.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Ptychobranchus fasciolara</i>	12.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Pyganodon grandis</i>	15	Ant/Pos	Midl/Pos	Ant/Pos
<i>Toxolasma parva</i>	2.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Toxolasma texasensis</i>	2.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Utterbackia imbecilis</i>	6.5	Ant/Pos	Midl/Pos	Ant/Pos
<i>Quadrula quadrula</i>	7	Ant/Pos	Midl/Pos	Ant/Pos
<i>Corbicula fluminea</i>	4	Ant/Pos	Midl/Pos	Ant/Pos
<i>Dreissena bugensis</i>	1.5	Bys/Pos	Bys/Pos	Bys/Pos
<i>Dreissena polymorpha</i>	1.5	Bys/Pos	Bys/Pos	Bys/Pos

Test #1: Feeding behavior when only the non-siphon anterior end was exposed to a food supply.

Initial test procedures were designed to isolate the inhalant and exhalant siphons of the unionids and *C. fluminea* from their food supply, to monitor for the production of pseudofeces and feces, or to observe the presence of algae in the stomach. Beakers containing water with algae (experimental) or without algae (reference) were covered with an impermeable latex membrane. The center of the membrane was cut so that the anterior end of the clam (non-siphon end) could be inserted through the slit into the beaker. The stretched membrane formed a tight seal around the clam's shell, preventing algae from leaving the beaker, but allowing the valves to partially open and the siphons extend. The clam and beaker were placed in an aquarium filled with filtered well water. After 30 minutes, the presence and location of pseudofeces were noted. Unionid clams were removed from the beakers, rinsed thoroughly, placed in individual containers filled with fresh well-water, and monitored for the production of fecal matter for the next 72 h. *C. fluminea* were dissected immediately after the 30-min test and stomach contents checked for the presence of algae. A total of 41 unionid specimens were tested: 4 individuals of each species (2 experimental and 2 reference) plus 1 *Quadrula quadrula* (experimental). A total of 10 *C. fluminea* were tested, 5 experimental and 5 reference. One experimental and one reference from each species were always tested at the same time. Unionids were not dissected to observe gut contents, but were reused for later tests.

Testing procedures for the dreissenids were modified to accommodate their rhomboidal shell shape and hinge position. Nonflagellated cultures of *C. reinhardtii* were used to form a mat of algae covering the bottom of a glass aquarium. The algal mat was covered by a

piece of plexiglass the size of the bottom of the aquarium. A small hole in the plexiglass allowed a dreissenid (experimental) to be placed ventral side down, in the opening. A second dreissenid (reference) was suspended near the test animal in a mesh cage, about one-cm above the plexiglass. After 30 minutes, the presence and location of pseudofeces were noted and all mussels dissected to check for algae in the stomach. A total of 50 *D. bugensis* and 50 *D. polymorpha* were tested, evenly divided between reference and experimental groups.

Test #2: Pedal feeding behavior on a benthic algal mat.

A second set of experiments was designed to determine if these freshwater bivalves were using some form of active pedal feeding. Unionids, *C. fluminea*, and dreissenids were placed upright on a mat of dried green algae covering a glass-bottomed aquarium and videotaped from underneath. A total of 37 unionids (2 of each species, except for 1 *Q. quadrula*), 4 *C. fluminea*, and 30 dreissenids were videotaped for at least one hour, and the location and appearance of all pseudofeces was noted. After the video taping, the unionids were rinsed, placed into individual containers, and monitored for the production of fecal matter for 72 hours. All *C. fluminea* and dreissenids were dissected immediately, and the stomach contents checked for algae.

Test #3: Feeding behavior when posterior siphon extension was prevented.

A third experiment was designed to determine if feeding would occur when the animal could not extend the siphons, thus preventing normal water-flow patterns inside the shell. The posterior part of the valves of *C. fluminea*, dreissenids, and unionids was taped to

prevent siphon extension. The experimental bivalves were placed upright on a mat of dried algae and videotaped from beneath the aquarium for at least one hour. Reference animals were not taped shut and treated similarly. All animals were then processed as described for test #2.

Normal siphon behavior

After non-siphon feeding tests, all unionid bivalves were returned to holding aquaria, where they were held upright in a gravel or sand substrate, or in open baskets, and fed planktonic live or dried green algae. These holding aquaria also contained Asian clams and zebra mussels. Random 24 hour videotaping of the animals in the aquaria was done weekly, with dim red lights used for nighttime filming. Videotapes were examined to determine the use of siphons under "normal" conditions. All animals videotaped were examined for the presence of pseudofecal and fecal release.

RESULTS

Test #1: Feeding behavior when only the non-siphon anterior end was exposed to a food supply.

All experimental unionids and Asian clams were able to obtain food presented only to the anterior end of the animal (Table 8). All unionids formed pseudofeces and fecal matter after the tests. All Asian clams had green algae in their mantle cavity, and in their stomachs. All 25 experimental *D. polymorpha* and 21 of the 25 *D. bugensis* that were placed with their byssus openings on the hole in the plexiglass formed pseudofeces and also contained algae in their stomachs. Four of the quagga mussels situated on the hole in the plexiglass formed no pseudofeces, nor did they contain algae in their stomachs or in their mantle cavities, indicating a lack of feeding. None of the reference animals, including the 50 dreissenids (both species) suspended in cages off the bottom of the test aquaria, or the unionids placed into beakers without algae formed pseudofeces (or fecal matter, unionids only). None of the reference specimens that were dissected had algae in their stomachs, indicating that algae were not accidentally present in the water column during testing. All of the reference animals and all of the experimental animals were observed to fully extend inhalant and exhalant siphons during testing.

Test #2: Pedal feeding behavior on the benthic algal mat.

All experimental unionids, both dreissenid species, and *C. fluminea* were able to obtain food from the algal mat as evidenced by the formation of pseudofeces and fecal matter after

the test was completed, or algae being present in the stomach (dreissenids and *C. fluminea* only) (Table 8). The reference animals from all of the species that were suspended in the water column did not show any evidence of food capture, either by their fecal production or direct examination of the stomach contents, indicating that planktonic algae were not present during the test. All experimental and reference specimens of all species studied, showed full extension of exhalant and inhalant siphons during the testing period.

Examination of the videotapes of all the animals placed on algal mats showed a consistent pattern in the removal of algae. The algal mat lost particles in a dendritic, random pattern, as algae streamed into the anterior portions of the unionids or *C. fluminea*, or into the byssus opening of the dreissenids. Algae were only removed from a certain radius underneath the shell, resulting in a clear spot being formed in the algal mat. Although food was captured by all of the experimental animals, none of the unionids or *C. fluminea* showed any active pedal feeding behavior as described in marine bivalves (Reid et al., 1992). In no instance did the foot actively sweep food particles into the mantle cavity; no pedal probing or pedal sweeping was observed. Unionids and *C. fluminea* did exhibit a consistent body position, with the anterior portion of the shell slightly open, and the edge of the foot slightly protruding at the shell margin. However, examination of the videotapes showed that none of these animals visibly moved the foot during the test.

In contrast, both dreissenids exhibited slightly different behavior. Examination of the videotapes indicated some foot movement, but the foot always remained inside the shell (in the mantle cavity) of the dreissenids that were exposed to the algae. There was no movement of the foot observed in the reference mussels that were not exposed to algae. No dreissenid was observed to extend the foot outside of the shell margin while being studied.

Test #3: Feeding behavior when posterior siphon extension was prevented.

Blocking the extension of the siphons did not prevent any of the experimental animals from capturing and ingesting algae. In addition, all of the non-taped reference animals were able to capture algae as well.

Normal siphon behavior

All bivalves that were returned to holding aquaria containing sand or gravel substrates and fed suspended algal food formed pseudofeces and fecal matter. This indicated that suspension feeding was occurring in all species tested. However, examination of the videotapes showed that none of the bivalves exhibited any other type of food gathering behavior by directed siphonal movements. We did not observe any deposit feeding or sand grain feeding either during day or night video recording.

Pseudofecal release

All species of unionids and *C. fluminea* were observed to release two types of pseudofeces (Table 8). One type of pseudofeces consisted of loose clouds of algae, lightly bound in mucus of apparently low viscosity, which was released from the anterior mantle edge, and from the inhalant siphon. Dense clumps of algae also were produced and the algae were contained in apparently high viscosity mucus. The pseudofeces composed of high viscosity mucus was almost exclusively released from the middle edge of the shell, anterior to the inhalant siphon. The lower viscosity clumps of algae were forcibly expelled from the bivalve, but the dense pseudofeces of high viscosity mucus remained in contact with or adhered to the shell, forming long strands of algae-laden pseudofeces. Both dreissenid

species released pseudofeces from the byssus opening as well as from the inhalant siphon. These pseudofeces contained algae entrapped in both low and high viscosity mucus. However, the higher viscosity pseudofeces were small in size, not long cohesive strands as were observed released by the unionids, and they rarely adhered to the shell.

DISCUSSION

Accessibility of Benthic Food Resources

These experiments demonstrated that benthic food resources are readily accessible to all of the adult dreissenids and unionids tested regardless of species, size, siphon type, or siphon extension and that the particle movement is best represented by the diagrams in Figures 14 and 15. The infaunal species (corbiculid and unionids) were as capable as the epibenthic species (dreissenids) of accessing benthic food supplies through either the anterior part of the shell or through the byssus opening. Furthermore, strong compartmentalization of feeding strategies and the array of specific feeding postures as commonly found in marine bivalves, where some species only siphon-fed or other species only foot-fed (Morton, 1960; Pohlo, 1969; Owen, 1974; Morton, 1983; McMahon, 1991; Reid et al., 1992) did not occur with the freshwater species while they were feeding on either benthic or planktonic foods.

The lack of specific body motion during benthic feeding, particularly the lack of pedal sweeping or substrate vacuuming by the inhalant siphon indicates that the food is being drawn into the mantle cavity by focused water currents. The dendritic appearance of the algal mat underneath these bivalves, as well as the limited distance that the algal mat was cleared, supports our hypothesis that focused water current is involved. This is probably similar to the anterior inhalant currents described by Reid et al. (1992) for juvenile marine bivalves and *C. fluminea* and for certain marine species such as *Polymesoda erosa* (Morton, 1996). Once inside the mantle cavity, the food particles appear to be accessible to the animal through normal food processing tracks.

Figure 14. (a) Diagrammatic representation of the uptake of food particles from the anterior (ANT) and posterior (POS) mantle regions by an infaunal unionid. (b) Release of high viscosity pseudofeces from anterior, middle (MIDL) and posterior regions by an infaunal bivalve. Inhalant siphon E, exhalant siphon, I.

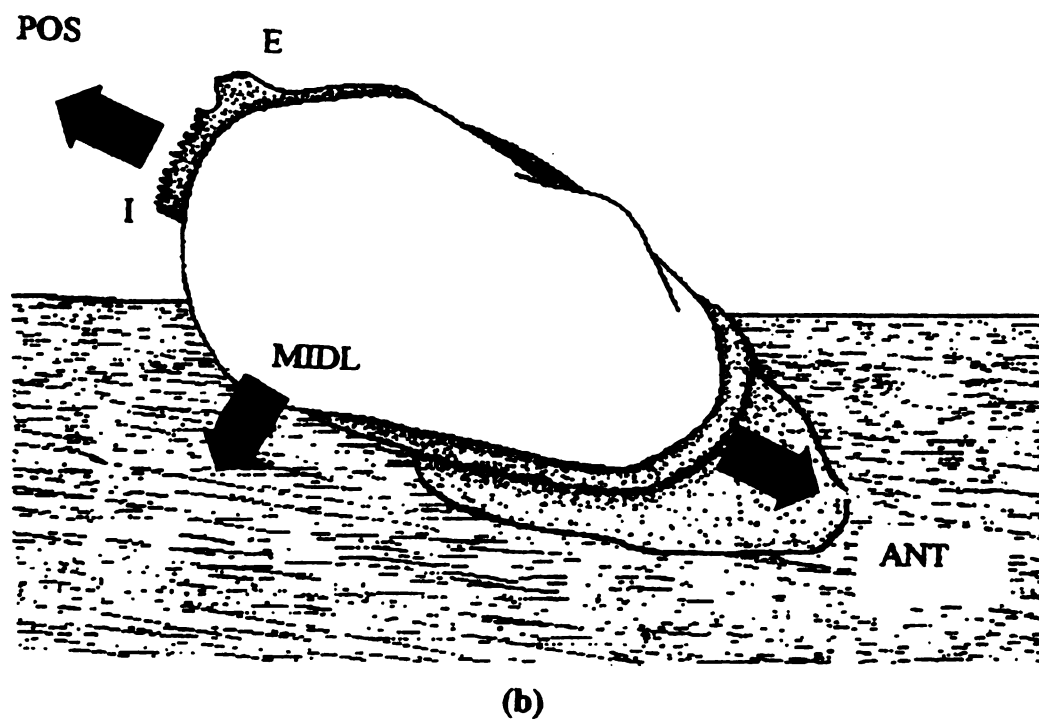
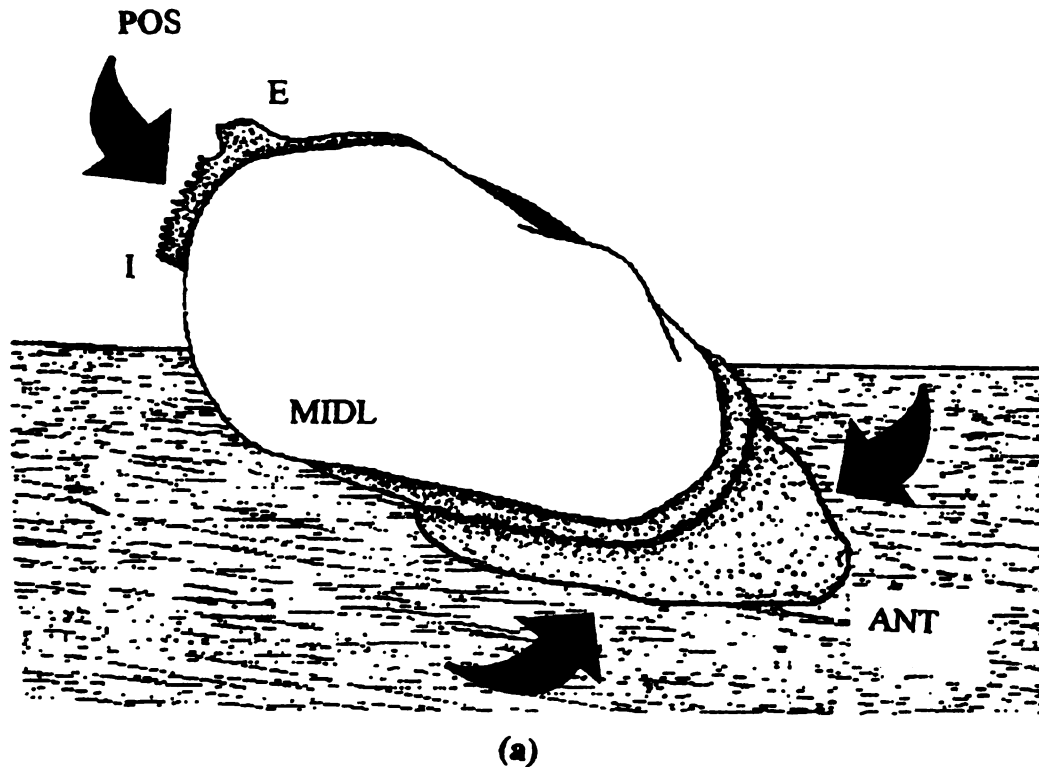
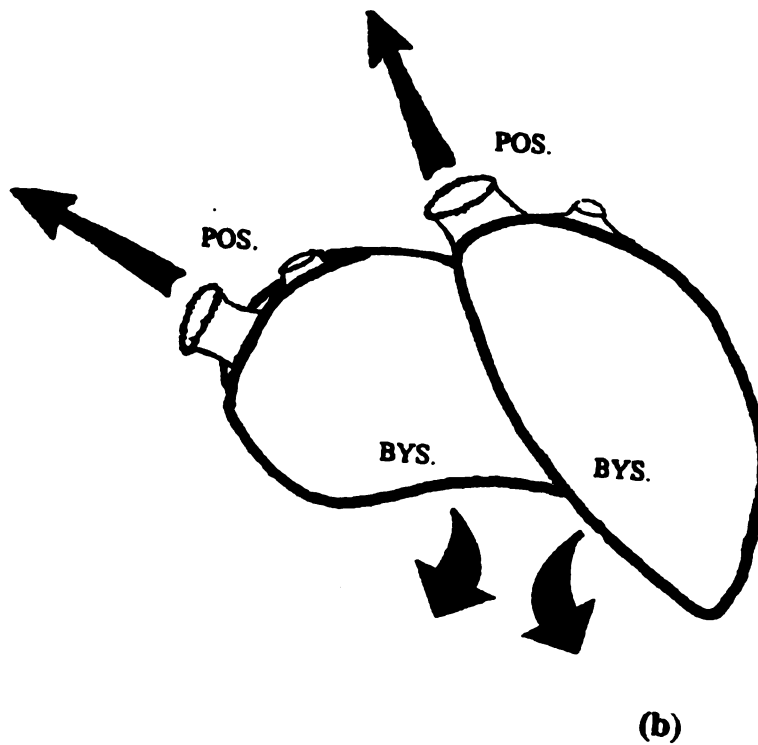
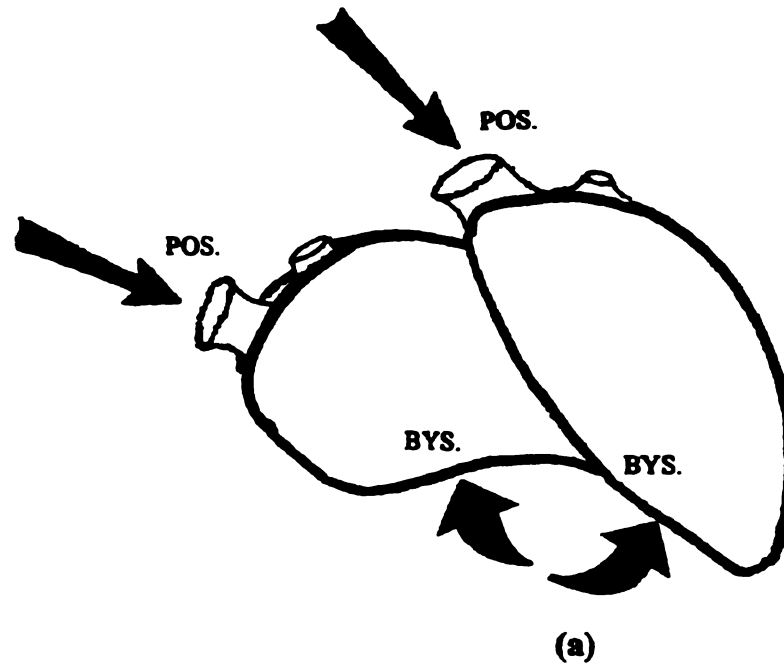


Figure 15. (a) Diagrammatic drawing of food uptake through posterior and byssus region of the mantle cavity by dreissenids. (b) Release of pseudofeces of both high viscosity and low viscosity through posterior and byssus region of the mantle cavity by dreissenids.



Ecological Significance

Theoretically, importance of benthic food supplies to the bioenergetics of unionid populations will obviously vary by season, site, species, and substrate. The limited distance that our experimental animals were able to clear a benthic mat of algae implies that there are spatial restrictions in the accessibility of benthic food resources by stationary, infaunal unionids. Way (1989) hypothesized that mobile unionid species are actually seeking and utilizing benthic food sources. However, as noted in Chapter 1 and in Raikow (1999) the diets of unionids from Four Mile Lake were dominated by benthic food resources while there was no evidence to indicate substantial mobility among these animals.

Benthic food supplies may be of more critical importance to colonial dreissenids, particularly to the more epibenthic *D. polymorpha*. *Dreissena polymorpha* colonies act as filter traps of organic debris, with pseudofeces and other particulate matter settling into the interstitial spaces in between mussels. This trapped organic matter supports a tremendous secondary benthic community, including amphipods, nematodes, protozoa and bacteria. Our experiments during this study indicate that zebra mussels have the ability to consume elements of this interstitial community, both through the water currents flowing into their inhalant siphons, and currents passing into the byssus opening (pedal gape feeding) (Figure 15). Such feeding strategies may be of primary importance to mussels located deep inside the colony, as they have little direct siphon contact with the surrounding water column.

Hypothetically, the accumulation of pseudofeces at the anterior/ventral shell margin may function as a food reservoir for these freshwater bivalves. This is easiest to visualize in colonial zebra mussels and, in fact, their ability to utilize non-suspended food resources may help explain the difficulties at some localities in correlating and modeling population

densities of *D. polymorpha* to such planktonic features as chlorophyll a, or secchi depth. The utilization of pseudofeces as food reservoirs by unionids and *C. fluminea* is more questionable since many of these populations live in flowing waters. The ability of all these bivalves to alter the location of pseudofecal release, either from the anterior, middle, or posterior part of the shell and to alter pseudofecal viscosity enhances the ability to use pseudofeces as food sources. Such would not be the case if the only location for the release of pseudofeces were from the incurrent siphon or if the particles were always so loosely mucused that they did not remain near the bivalve.

All our test bivalves produced both heavily mucused and lightly mucused particles from anterior as well as posterior shell margins. Beninger and St. Jean (1997) have postulated that particles were covered with highly viscous mucous before release as pseudofeces by marine bivalves in order to prevent the particles from reentering gill cilia sorting tracks. In our experiments, it was the highly-mucused pseudofeces that upon release remain close to the animal and are accessed later. The lightly mucused particles are more commonly expelled from the inhalant siphon and drift some distance away. We are unable to determine what stimulus triggered the production of either lightly- or heavily- mucused particles.

CONCLUSIONS

Based on the previous experiments, the statistical and research hypothesis are rejected for the following reasons:

~~$H_0: \mu_1 = \mu_2$. Water intake into the bivalve is limited to the inhalant siphon.~~ This hypothesis was rejected and the alternate hypothesis accepted, as water entered the bivalve shell anteriorly, posteriorly, and ventrally.

~~$H_0: \mu_1 = \mu_2$. Water outflow into the unionid is bi-directional through the inhalant siphon and unidirectional through the exhalant siphon.~~ The null hypothesis was rejected and the alternate hypothesis accepted as the exhalant and inhalant siphons were not the only site of water outflow from the shell.

~~$H_0: \mu_1 = \mu_2$. Water intake and outflow patterns differ between *C. fluminea*, *D. polymorpha*, and all the unionid species.~~ The null hypothesis was rejected and the alternate hypothesis accepted, as there was no difference in ability to access food or water from a number of locations on the shell of all bivalves tested.

Most freshwater bivalves are opportunistic in their modes of feeding, capable of producing focused water currents from anterior mantle margins as well as posterior locations in order to access planktonic, benthic, and within sediment, food supplies. The ability to shift feeding strategies dependent on food supply would explain the variability in modes of feeding seen in different studies. We hypothesis that pseudofeces can actually serve as a

food reservoir for some bivalves, particularly the epibenthic, colonial *Dreissena polymorpha*. Further research is needed on behavior of bivalves under field conditions to determine what proportion of benthic or planktonic foods are actually ingested on a seasonal basis by particular bivalve species.

CHAPTER 4

Substitute Diets for Live Algae in the Captive Maintenance of Adult Freshwater Unionidae.

Nearly 70% of the freshwater Unionidae in North America are currently facing extinction due to the combination of chemical pollution, habitat alteration, and the invasion of non-native species such as the zebra mussel. Conservation efforts have focused on a number of areas, including relocation of endangered populations, and aquaculture of recently transformed larvae. While captive maintenance of endangered animals is a common technique used to enhance and preserve species-at-risk, freshwater unionids have proved to be difficult to maintain in captivity. To date, most aquacultural efforts have concentrated on developing live algal diets that will support the growth and survival of larvae or juveniles (≤ 1 year of age). Recent research has developed a tri-algal diet that appears to support some survival of a few of the species tested (see Gatenby, et al 1996; Gatenby, et al 1997). However, adult bivalves have rarely been kept alive for more than three years even in hatchery-type ponds that are fertilized to maintain algal production. One problem in maintaining adult unionids is the lack of information regarding the actual nutritional requirements of these animals for growth and reproduction.

As presented in Chapter One, the dietary habits and bioaccumulation patterns of some unionid species indicate that these animals rely more on bacteria as a nutritional source and accumulate vitamin B₁₂ and cholesterol. In Chapter Two, we showed that unionids do not

retain bacteria capable of digesting cellulose or chitin, they have to use transient fauna in order to initiate digestion of these complex polysaccharides. We have taken the information learned regarding field populations and tested a variety of diets presently available on the open market for other invertebrate filter-feeders as well as generated diets, in order to determine the feasibility of using non-live algal- based diets to support adult unionid growth, reproduction, and survival under captive conditions.

The statistical and research hypotheses based on these objectives are as follows:

1. $H_0: \mu_1 = \mu_2$ Unionid adults require live algae to support growth and survival.
2. $H_0: \mu_1 = \mu_2$ Unionid adults require live algae to support reproduction.
3. $H_0: \mu_1 = \mu_2$ Unionid juveniles require live algae to support growth and survival.
4. $H_0: \mu_1 = \mu_2$ All unionid species respond the same to substitute diets.

METHODS

We tested various feeds on freshwater unionids from 1994 to 1998. Eight unionid species were involved in the tests- *Amblema plicata*, *Cyclonais tuberculata*, *Lampsilis fasciola*, *Lampsilis ventricosa*, *Lampsilis siliquoidea*, *Leptodea fragilis*, *Pyganadon grandis*, and *Quadrula quadrula*. These animals were selected due to their ready availability and due to the need to maintain large numbers of unionids for an extended period of time during the salvage operations at one of our study sites, Metzger Marsh (see Nichols and Wilcox 1997). *Amblema plicata*, *Leptodea fragilis*, *Pyganadon grandis*, and *Quadrula quadrula* were obtained during rescue operations at Metzger Marsh, a coastal wetland in western Lake Erie. *Cyclonais tuberculata*, *Lampsilis fasciola*, *Lampsilis ventricosa*, and *Lampsilis siliquoidea* were collected from a small, regulated stream (Huron River) currently being colonized by zebra mussels. One hundred adult unionids (at least 3 years of age according to external annuli) of each species were used in the initial tests on diet acceptance and survival, with the exception of *C. tuberculata* (15 total) and *L. fasciola* (15 total). All animals were measured (shell height, length, and width) immediately upon arrival in the laboratory and assigned an individual tracking number which was etched into one of the valves.

The ability of the various diets to support the increase in shell growth (height, length, and width) was based on the growth of a number of $\leq$ three-year-old individuals of some species that became available in the fall of 1996. The number of young unionids varied by species, *Amblema plicata* (75), *Cyclonais tuberculata* (15), *Lampsilis fasciola* (2), *Lampsilis ventricosa* (10), *Lampsilis siliquoidea* (2), *Leptodea fragilis* (100), *Pyganadon grandis* (100), and *Quadrula quadrula* (50). The size of these animals varied from 10-30 mm in shell length and we will refer to them as juveniles. As with the mature adult unionids, these

young unionids were measured upon arrival at the laboratory and identification numbers assigned (written on the shell with waterproof marker).

Both adult and juvenile unionids were held at the Great Lakes Science Center in three different types of rearing systems. First, randomly selected adult (1/2 the total number) and juvenile (1/3 of the total available) unionids (1/2 of total for the adults and 1/3 of the total) of each species were placed in a series of individual glass aquaria (4L), with static water flow conditions. Airstones were introduced to provide water circulation and to increase oxygen levels. These aquaria were stripped and cleaned three times weekly. Second, randomly selected adults of each unionid species (1/2 of total) plus 1/3 of the juveniles were placed in a series of 600L rectangular troughs, containing 10 cm of coarse gravel. This was a flow-through system, with a water replacement rate of 4L/h. Airstones were placed every 100cm along the trough length.

Third, we used a series of 5 upwelling chambers (2L each) based on systems used for rearing marine bivalves. In these chambers, water flowed up through the coarse gravel substrate, across the unionids and then out the top of the chamber at a rate of 4L/h. This flow was restricted to 4x/day, for 15 m. The upwelling chambers were mainly used with the juvenile unionids. One third of the juveniles for each species were randomly selected and placed in these chambers.

The water used for all rearing systems was identical. We used well water with an initial CaCO_3 concentration of $\sim 100\text{mg/L}$ as measured using the EDTA titrimetric method (APHA 1989), a dissolved oxygen content of 8.0ppm using the Winkler method of measurement (APHA 1989), a dissolved ammonia concentration of $<0.5\text{ppm}$ using the phenate method of measurement (APHA 1989), and a pH of 7.8 using a Fisher Scientific Accumet pH meter

model #AB15. Water quality parameters were measure weekly and during any die-off of unionids. Water temperature averaged 15° C, and the light regime was on a 12 light/12 dark cycle.

A total of 10 different diets were tested for their ability to support survival, growth and/or reproduction of the adult and juvenile unionids. These diets were selected based on their ready availability, utilization in marine bivalve culture (e.g. marine algal pastes, bacterial slurries) or some often reported anecdotal beliefs that unionids would feed and “survive” on a particular product (e.g. fish flake food). Five of the diets were commercial, products that only required mixing with water before feeding- dried chlorella (Earthrise Co., California, USA), marine algal paste (Coastal Oyster, *Thalassiosira pseudonana*), rotifer replacement diet (Hatchfry Encapsulon, 30µ size particles, Argent Co. Washington, USA), dried fish flake food (tropical fish), and a manipulated yeast diet (Artemia Reference Center, Ghent Belgium). The other 5 diets were experimental. The first experimental diet a microencapsulated feed with feed particles imbedded in a gel matrix concocted according to Langdon’s (Langdon and Levine, 1983; Langdon and Bolton, 1984; Buchal and Langdon, 1995) work on marine bivalves (referred to as Langdon’s recipe). The second experimental feed consisted of dried whole egg (60% by weight), dextrin (30%), safflower oil (9%), and vitamins, mixed for 10 m at 35000 rpm just prior to feeding (referred to as egg chow). The nutritional proportions of this diet are based on the proximate analysis data for freshwater bivalves (Secor et al., 1993).

The third experimental diet of a live bacterial/ciliate slurry was based on Stuart’s (1982) work on the marine mussel *Aulacomya*. This feed is made by taking finely ground vegetation ($\leq 20 \mu$) that is allowed to soak, and develop bacterial and ciliate communities

(rot), for three days before feeding. Stuart used kelp as the vegetation base; we used freshwater marsh grasses. The fourth experimental diet was a mixture of 50% bacterial/ciliate slurry (#2) and 50% dried chlorella. The fifth experimental diet was a mixture of 30% bacterial/ciliate slurry, 30% dried chlorella, 10% artemia enrichment supplement (lipids and algal growth enhancers, Rich Advanced from Sanders Corp. Ogden Utah), a 10% dried invertebrate enrichment supplement (lipids and algal growth enhancers, Sanders Black Gold, from Sanders Corp., Ogden Utah) and 20% dextrose (bacterial/ciliate slurry #3). The chlorella was added to enhance the carotenoid level (see Cowey and Tacon, 1982), cholesterol and phytosterols to raise the sterol level (see Teshima ,1982) and dextrose as an easily digestible energy source for bacteria, ciliates, and unionids.

The feeding rate of all diets was geared toward a 5-8 mg dry weight/L of aquaria water for at least 15 hours out of the day. This rate is based on the average total organic particulate matter values found in the Huron River near a large free-living unionid bed as described in Chapter 1.

Measuring Success of Diet Formulations

We looked at the ability of the various diets to support survival, maintenance of body weight, the formation of glochidia, and to a lesser extent growth in adult unionids. Growth as measured by increase in shell length, height, or width, was not considered a major factor due to the age of the animals being tested. Survival was monitored daily. Growth was monitored every 3 months, or when the animal died. Glochidial formation was checked monthly by visual examination of the gill chambers in known females. Any animal that died during testing was autopsied and the physical appearance of internal organs noted. Any

obvious lesions were dissected and examined for flukes, fungus, or bacteria. The digestive gland and reproductive track were always dissected, a fresh tissue mount prepared, and examined under a compound microscope, to assess changes in gross structure and epithelial layer.

Growth and survival were considered the most important factors in measuring the suitability of the various diet formulations for the juvenile unionids. Each individual unionid was measured monthly and survival checked daily.

The statistical relationships between differences in survival rates of adult and juvenile unionids on the various diets was determined using analysis of variance (ANOVA) followed by a Tukey-Kramer procedure to take into account the unequal replicate sizes, particularly with the juveniles. Differences in growth rates between juveniles of different species on different diets were analyzed using analysis of covariance (ANCOVA), followed by a sequential analysis of the slopes. Results were considered different at the $p \leq 0.05$ level. Treatments without survivors were not included in any of the analyses.

RESULTS

Of the ten diets tested on adult unionids, none could be recommended without reservation, although some do show potential for use in captive maintenance. Two of the diets were rejected almost immediately, six supported growth and survival for at least one year, and three supported glochidial formation (Table 9). All caused problems with water quality and some mortality was more directly related to rapid changes in water quality than to diet. Initially, all the unionid species ingested all of the ten diet formulations. However, two of the diets, the commercial marine algal paste and the experimental encapsulated feed (Langdon's recipe) caused severe stress in all the animals tested; the marine algal paste caused excessive mucous formation, which was detrimental to water quality parameters and the encapsulated feed caused gaping. Both diets were dropped from the tests within one month. A third diet, the Hatchfry Encapsulon, was dropped from the tests after seven months. Hatchfry Encapsulon, a commercial rotifer-replacement feed, was initially very successful. All the unionid species fed well on it, and grew rapidly, up to 4 mm in the first three months. Unfortunately, when we ordered the second batch of feed, the unionids refused to eat it (as determined by the lack of fecal matter). We reordered the feed, but the unionids continued to refuse the feed. After one month this diet was dropped from the tests.

Two of the three remaining commercial feeds, the fish flake food and the manipulated yeast, could not support survival of any unionid species for longer than 13 months (Tables 10 and 11). None of the unionids showed any shell growth at all while on these diets. The third commercial feed, the dried *Chlorella* did better, but still did not support long-term survival or continuous growth. All the species fed *Chlorella* survived at least 15 months and

Table 9. Synopsis of ability of various diets to support growth, survival, and reproduction of both adult and juvenile unionids.

	Dried <i>Chlorella</i>	Marine algal paste	Hatchfry encapsulo	Fish Flake Food	Yeast	Encap- sulated	Egg chow	Bacterial slurry #1	Bacterial slurry #2	Bacterial slurry #3.
Ingested	+	+	+	+	+	+	+	+	+	+
Ingested but caused stress		+				+				
Survived at least 6 months	+		+	+	+		+	+(1)	+	+
Survived ~ 1 year	+		+	+			+	+	+	+
Survived ~2 years							+	+	(3)	(2)
Survived ~3 years							+	(3)	(3)	(2)
Showed shell growth	+		+				+	+	+	+
Initiated glochidia							+(4)			+

- (1). Kills *Pyganadon grandis* almost immediately.
(2). Tested only for one year.
(3). Tested only for two years.
(4). 20% of females died after glochidial release.

Table 10. Adult unionid survival at 12 months on various diets.

	Unfed	Dried <i>Chlorella</i>	Hatchfry Encapsulo	Fish Flake Food	Yeast	Egg chow	Bacterial slurry #1	Bacterial slurry #2	Bacterial slurry #3
<i>A. plicata</i>	45%	100%	51%	25%	100%	100%	100%	100%	100%
<i>C. tuberculata</i>	*	*	*	*	*	100%	*	100%	100%
<i>L. fasciola</i>	*	*	*	*	*	0%	*	0%	0%
<i>L. ventricosa</i>	21%	100%	14%	5%	2%	100%	3%	100%	100%
<i>L. siliquouidea</i>	15%	100%	11%	10%	1%	100%	4%	100%	100%
<i>L. fragilis</i>	12%	100%	6%	0%	0%	100%	0%	100%	100%
<i>P. grandis</i>	32%	100%	37%	0%	0%	100%	0%	100%	100%
<i>Q. quadrula</i>	0%	100%	54%	0%	79%	100%	100%	100%	100%

* indicates diet not feed to that species.

Table 11. Adult unionid survival at 24 months on various diets.

	Unfed	Dried <i>Chlorella</i>	Hatchfry Encapsul	Fish Flake	Yeast	Egg chow	Bacterial slurry #1	Bacterial slurry #2	Bacterial slurry #3
<i>A. plicata</i>	0%	0%	0%	0%	0%	87%	78%	82%	*
<i>C. tuberculata</i>	*	*	*	*	*	89%	*	0%	*
<i>L. fasciola</i>	*	*	*	*	*	0%	*	0%	*
<i>L. ventricosa</i>	0%	0%	0%	0%	0%	78%	0%	0%	*
<i>L. siliquouidea</i>	0%	0%	0%	0%	0%	71%	0%	0%	*
<i>L. fragilis</i>	0%	0%	0%	0%	0%	70%	0%	0%	*
<i>P. grandis</i>	0%	0%	0%	0%	0%	72%	0%	0%	*
<i>Q. quadrula</i>	0%	0%	0%	0%	0%	75%	80%	77%	*

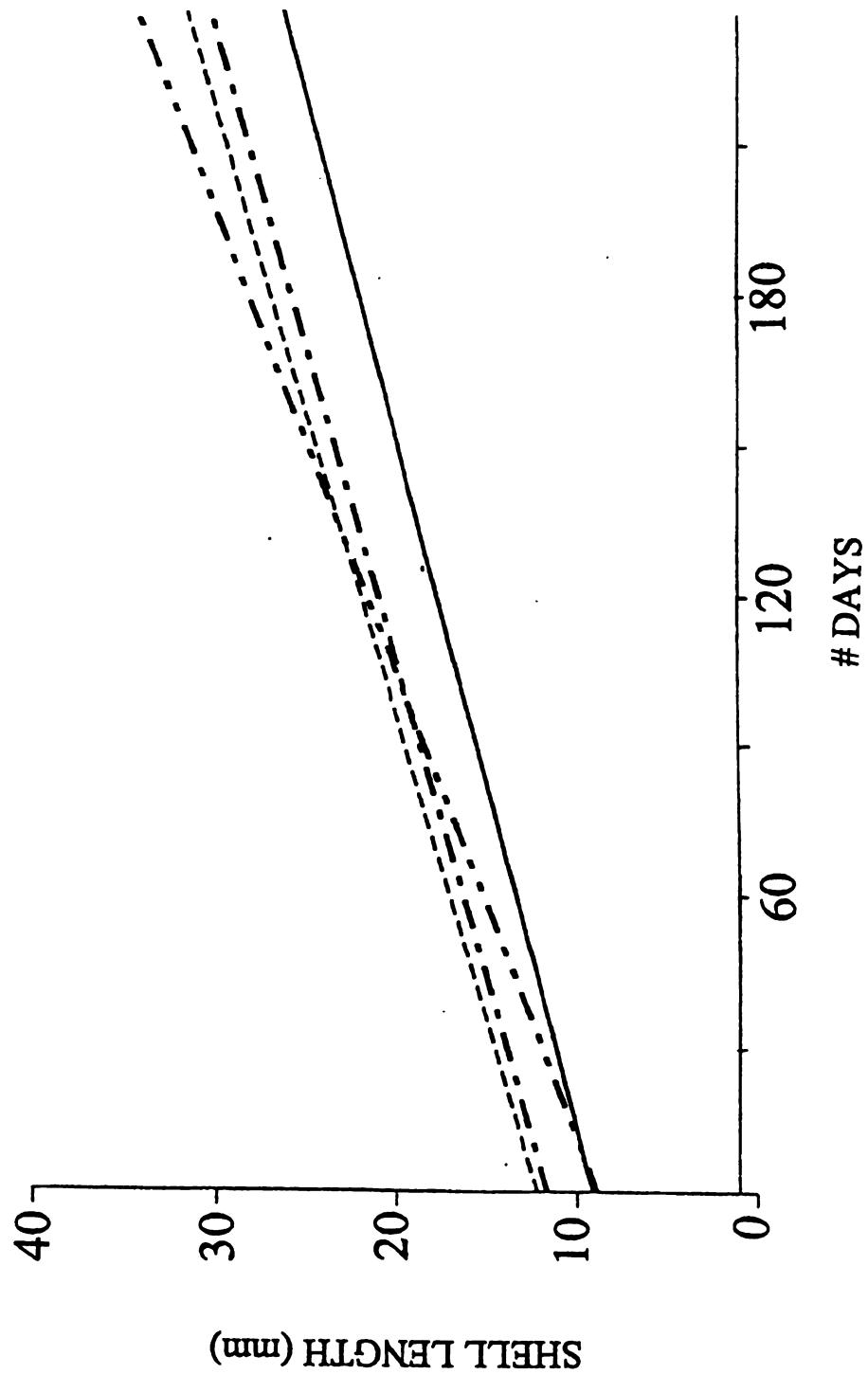
* indicates diet not feed to that species.

showed shell deposition during the first 6 months, but not afterwards. There was no significant difference in growth rate of any unionid species on either diet and the maximum growth shown for the 13 month period was 2.75 mm (seen in the first 6 months).

The experimental diets, with the exception of Langdon's recipe, were successful in supporting unionid survival and growth, but long-term survival (>3.5 years) was still problematic (Tables 9-11). The high-protein egg chow was one of the best diets for supporting survival, growth and reproduction of both adults and juveniles, at least up to year three. Adult survival up to year two was 70% or greater for seven of the eight species. The exception, *L. fasciola* did not survive on anything. At the beginning of year three, the survival rates of the adult unionids were still high: *A. plicata* 81%, *C. tuberculata* 80%, *L. ventricosa* 72%, *L. siliquoidea* 65%, *L. fragilis* 64%, *P. grandis* 71%, and *Q. quadrula* 69%. Twenty-one percent of the *P. grandis* and 5% of the *L. fragilis* females formed glochidia during year 2, while on this diet. None of the thick-shelled species formed glochidia. However, during the third year on this diet, the adult unionids began to die and by year four, all had perished. The body weight of these animals was high, indicating that starvation was not a factor. Autopsies showed no signs of parasitism or other disease factor, but all of these animals had greatly enlarged kidneys. Kidneys were over 100% larger than normal.

This diet did support growth in all the juvenile unionids tested although the amount of shell deposition varied significantly according to species. The thin-shelled species grew the most over the 350-day period, with *P. grandis* showing an average increase in shell length of 8.7 mm, *L. fragilis* at 8.1 mm. The thick shelled species (*A. plicata*, *C. tuberculata*, and *Q. quadrula*) grew significantly less, averaging 6mm. The growth equations are presented in Figure 16.

Figure 16. Comparison of growth equations of various unionid species on experimental egg chow over a period of 280 days. Q= *Quadrula quadrula* ($Y = 9.1 + 0.801X$ $R^2 = 0.87$). A= *Amblema plicata* ($Y = 11.6 + 0.657X$ $R^2 = 0.83$). P= *Pyanadon grandis* ($Y = 12.2 + 0.733X$ $R^2 = 0.85$). L= *Leptodea fragilis* ($Y = 8.8 + 0.951X$ $R^2 = 0.98$).

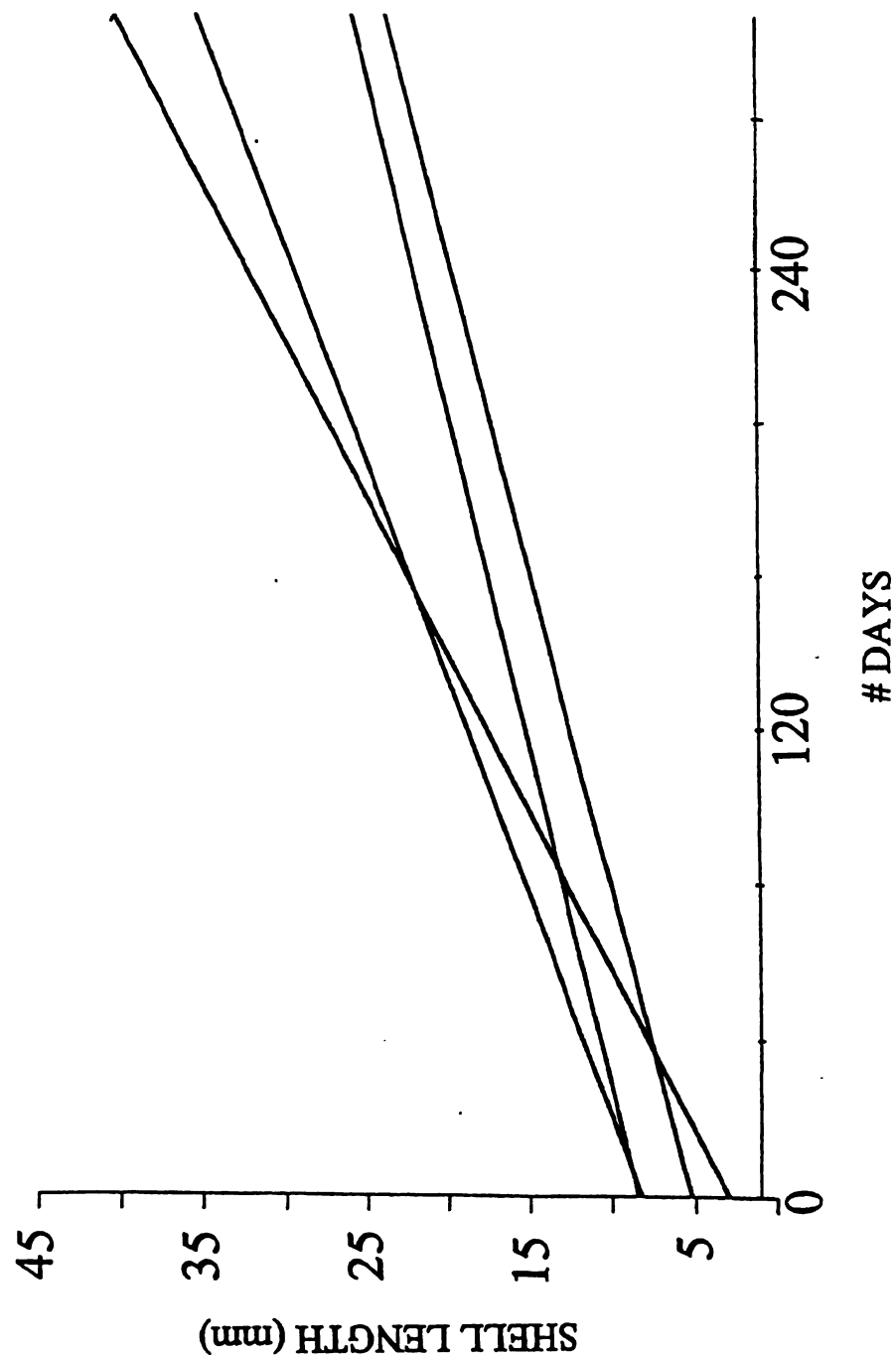


The other experimental diets, the various versions of bacterial slurries differed in their ability to support unionid growth and survival. The first version, bacterial/ciliate slurry #1 (based on Stuart, 1982) proved an acceptable feed for species such as *A. plicata* and *Q. quadrula*, but killed *P. grandis* within a day or two of the initial feeding and the *Lampsilis* species within a few weeks. *Amblema plicata* and *Q. quadrula* adults survived well on this diet during the two years it was tested, but no reproductive effort was seen (Table 11). Young juveniles of these two species grew an average of 4mm, less than on the egg chow or the other version of this diet.

The bacterial/ciliate slurry formulation #2, which contains dried chlorella provided the same basic results regarding survival and growth as was seen with the bacterial/ciliate slurry #1. *Pyganadon grandis* and none of the *Lampsilis* species could not tolerate this feed, but once again, *A. plicata* and *Q. quadrula* did well. Growth rates of juvenile *A. plicata* and *Q. quadrula* were not significantly different than seen in with the bacterial/ciliate slurry #1.

The final experimental diet formulation, the basic bacterial/ciliate slurry #3 with the addition of various lipid supplements was acceptable to all the species tested and provided the greatest amount of growth seen in any of the diets. *Pyganadon grandis* did well on this diet, as did all the other unionid species tested. Survival of the adults was at 100% after one year, with the exception of *L. fasciola*, (*Lampsilis fasciola* died regardless of what they were fed or how they were handled). The juvenile unionids grew an average of 7.1 mm for *A. plicata*, *C. tuberculata*, and *Q. quadrula* and 9.5 mm for *L. fragilis* and *P. grandis*. The growth equations for this group of juveniles is presented in Figure 17. Unfortunately, we tested this diet only for one year and have no long-term survival data.

Figure 17. Comparison of growth equations of various unionid species on bacterial slurry #3 (added lipids) over a period of 350 days. Q= *Quadrula quadrula* ($Y = 8.4 + 0.789X$ $R^2=0.99$). A= *Amblema plicata* ($Y = 5.2 + 1.003X$ $R^2=0.93$). P= *Pyanadon grandis* ($Y = 2.9 + 1.255X$ $R^2=0.99$). L= *Leptodea fragilis* ($Y = 8.2 + 0.999X$ $R^2=0.99$).



Water quality was difficult to maintain on all types of holding aquaria. There were 22 episodes of die-off due to problems with water quality during the four years of tests. The worst episodes occurred in the static aquaria, followed by the flow-through troughs, but two events occurred in the upwelling systems. During these events, dissolved oxygen levels would plummet to <1ppm and ammonia levels rise to >3ppm often in less than 12 hours. Both of these events involved pump failures.

DISCUSSION

The problem in evaluating diets is that although a number of our diets supported growth and short-term survival (≤ 3 years), few of the unionids survived for longer than 3.5 years. There is no way to factor out problems relating to dietary factors from those potentially related to environment or from disease. However, we can make some inferences regarding unionid captive care based on our experiences over the last few years and from nutritive data recently obtained from field populations. First, unionids are obviously omnivorous and will eat both high protein, low fiber feeds such as the egg chow, as well as low protein, high fiber feeds such as the bacterial/ciliate slurries. We no longer recommend using high protein feeds of any kind for these animals, even though they are readily ingested. Our decision is based first on the fact that field populations of unionids in the Huron River were feeding on a low protein-high fiber food source (see Chapter 1). Secondly, although our high protein egg chow supported growth, limited reproduction, and short-term survival, it did not support long-term survival and the dying animals showed greatly enlarged kidneys. Feeding excessive dietary protein to vertebrates that normally utilize low protein feeds has been known to cause high mortality due to kidney failure after a few years. However, unionid kidneys do not function in ammonia excretion; that function is performed by the gills. Unionid kidneys primary function is to control ionic balance of bodily fluids. Theoretically, excess protein might affect ionic balance due to alteration of blood pH due to excess amines. However, enlarged kidneys may also be due to some long-term sublethal environmental or dietary problem. High protein is not a dietary requirement of unionids since these bivalves grow as well on low protein feeds (bacterial/ciliate slurry #3) as on a high protein feed. High protein diets are not recommended for long-term use.

To date, the most successful diet we tested was the low protein bacterial/ciliate slurry #3 with the added lipids. This diet was the closest in content to the food resource utilized by wild unionids in the Huron River (Chapter 1). It proved acceptable to all species tested, supported growth in the juveniles and kept the animals alive for one year. The reduction of the bacterial component from the 50% levels used in bacterial/ciliate slurry #1 permitted the survival of *P. grandis*. The addition of extra lipids, particularly cholesterol, increased the growth rate from that seen with bacterial/ciliate slurry #1 & #2. The cholesterol was added because our analysis of the food fraction being utilized by the unionids in the Huron River showed that cholesterol was the dominant lipid class and that the unionids themselves were accumulating cholesterol in higher densities than other lipid classes.

The question remains as to whether freshwater unionids can make cholesterol from shorter chain fatty acids, dealkylate phytosterols to form cholesterol, or must obtain cholesterol from their diets. Various studies have examined lipid content of a few species of unionids over a seasonal basis, but have not postulated on the origin of this lipids. The question regarding lipid requirements have not been fully answered for commercially valuable marine species, such as oysters, although C22 and C223 lipids are hypothesized as critical (see Teshima, 1982). Certainly the addition of cholesterol to the diet formulation did result in a significant increase in growth rates but due to addition of carotenoids, may not be sole reason. Although the diet formulation #3 was successful in supporting growth and survival, the main problem is that we only tested this diet for one year. As our experiences with the egg chow show, one-to-two year tests may lead to inaccurate interpretation on a diet's suitability for long-term captive maintenance. This is not a problem limited to non-algal diets. Experiences with algal diets on adult unionids show similar problems with

maintenance after 3 years (e.g. Gatenby et al., 1997).

This mortality after three years in captivity may not be a nutritional problem at all. Sublethal water quality or environmental parameters may result in subliminal stress levels that eventually kill the adult unionids. One of the greatest concerns is that of maintaining water quality. We experienced 22 different episodes over 3.5 years where short-term water quality problems caused unionid mortality. Usually these episodes were directly related to well pump failure, or a ruptured pipe reducing water flow. Water quality problems with non-algal feeds especially with bacterial/ciliate slurries would be expected to be greater than with live algal feeds. However, Gatenby et al. (1994; 1996) used live algae for a food supply and still reported mortality incidents due to water quality problems. In our study, water quality was easier to maintain at acceptable levels in the upwelling system, than in the flow-through or static aquaria.

Other environmental factors may also be responsible for unionid mortality after a number of years. For example, one potential problem in unionid care under intensive aquaculture facilities is the lack of natural temperature regimes. Most unionids under field conditions undergo a period of winter chilling that is not usually the case for animals in laboratories where the animals are usually maintained. Lack of shifting temperature regimes has been known to cause mortality in other types of animals such as the Mediterranean tortoises mentioned earlier. Such subnormal environmental factors need further study before we can establish captive management protocols for the various unionid species. There will be some species variability in environmental tolerances. For example, we were never capable of keeping *L. fasciola* alive in any type of environmental condition, even though we

were able to keep companion species removed from the same area of the Huron River alive for several years.

A further cause of problems on various dry diets is that, as seen in Chapter 2, unionids do not retain a true endemic flora capable of digesting cellulose or chitin. This means that dry diets that are based on these food items must undergo a period of bacterial predigestion before being used as feed. This lack of appropriate microflora also occurs in oysters (e.g., Garland et al., 1982), which also do poorly when fed dry diets (Coutteau and Sorgeloos, 1993).

CONCLUSIONS

Based on the experimental tests of the various diets, the statistical and research hypotheses were rejected and the alternative hypotheses accepted:

$H_0: \mu_1 = \mu_2$ Unionid adults require live algae to support growth and survival. Null

hypothesis rejected because adult unionids lived for three years and grew on diets that contained no live algae.

$H_0: \mu_1 = \mu_2$ Unionid adults require live algae to support reproduction. Null hypothesis

rejected because adult unionids did reproduce on diets containing no live algae.

$H_0: \mu_1 = \mu_2$ Unionid juveniles require live algae to support growth and survival. Null

hypothesis rejected because juvenile unionids survived and grew on diets that contained no live algae.

$H_0: \mu_1 = \mu_2$ All unionid species respond the same to substitute diets. Null hypothesis

rejected because unionids differed in their ability to survive, grow, and reproduce on the various diets tested.

Long-term studies (>3 years) are needed to accurately assess captive management protocols and diets. Growth, survival, and even reproductive efforts over shorter periods of time may not guarantee survival after three years. Based on our research, most of the mortality appears related to sudden loss of water quality and to improper diets. Adult

unionids can be maintained on non-live algal diets with the following caveats. First, water quality is critical and very difficult to maintain when any non-living diet is fed. Upwelling systems appear to be the most reliable for long-term care. A number of non-live algal diets appear suitable, but corn or soybean based dry feeds with some bacterial pre-digestion to insure access to cellulose or chitin molecules were most successful. Any diet formulation should contain vitamin B₁₂ and additional cholesterol. Further work is needed to determine exactly what conditions are causing mortality even in animals that are growing and reproducing.

SUMMARY

The feeding habits and nutritional requirements of North America's Unionidae are integrally interwoven with the microbial loop and while the utilization of such an ubiquitous food resource permits colonization of many different types of habitats, it makes captive management of these animals very difficult. Unionids in the Huron River are feeding on small particle FPOM, which is high in detritus and low in protein. Microbial carbons and microbially-derived vitamins dominate the soft tissue stores of the unionids. Microbes are such an important part of the diet that some species of unionids do not appear to be functioning as primary consumers. The $\delta^{15}\text{N}$ ratios that unionid species function as both primary consumers, and omnivores, and possibly secondary consumers. One factor that needs further research is the role of microbial exopolymers in unionid nutrition. Further information is also needed on the role that the invertebrate community inhabiting the mantle cavity of the unionids plays in food predigestion and exopolymer production.

Unlike most animals that ingest large quantities of detritus, unionids do not rely on endemic microbial fauna for food digestion or food resource partitioning. Only one microbe was found that appeared to be endemic and it occurred in all species from all habitats. Cellulolytic and chitinolytic microbes were transient, present only when the unionids were feeding, and were habitat, not species specific.

Applying the feeding information obtained from field studies to captive management of adult unionids highlights the difficulties of maintaining filter-feeding invertebrates in captivity. The field data would indicate that the unionids need a food source $<28\mu$ in size, low in protein, low in algae, and high in microbes of various types, particularly cellulolytic and chitinolytic types. This food must be provided at low levels ($<5\text{mg/L}$) on an almost

continuous basis. The fastest growth and best survival over the test period was seen on an experimental diet that blended FPOM, dried algae, and nutrients such as cholesterol and vitamin B¹². Water quality problems occurred frequently, due to the inclusion of live microbes in the diets. One rearing system, the upwelling chambers, proved most effective at minimizing water quality problems. Many of the diets tested were effective at supporting some level of survival and growth for short periods of time (<3 years) but proved ineffective and supporting survival for longer periods of time. Further research is needed to refine nutritional needs and microhabitat requirements in order to reduce any sublethal environmental and dietary problems that may be inducing health problems after several years in captivity.

FUTURE RESEARCH NEEDS

Conservation of unionid resources in North America must achieve a higher level of success with the adult stages than is presently occurring. Increasing our ability to successfully manage these bivalves will require further testing of diets and holding facilities for captive populations as well as increasing the amount and complexity of studies on feeding habits and environmental requirements of field populations. The Huron River and Four Mile Lake represent typical streams and lakes found in northern temperate areas, but do not represent highly productive streams and lakes in the southern United States where most of the rare unionid species are located. Future research needs to focus on identifying feeding habits in field populations of as many species as possible, under as many different conditions as possible in order to factor out obligate versus opportunistic feeding behaviors. It is critical that these efforts proceed as rapidly as possible as so many unionid communities are decreasing in diversity and abundance, permanently altering their relationship with the local food web and habitat.

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