This article is Contribution 999 of the USGS Great Lakes Science Center.

Clearance of laboratory-cultured bacteria by freshwater bivalves: differences between lentic and lotic unionids

Harold Silverman, S.J. Nichols, Julie S. Cherry, Eric Achberger, John W. Lynn, and Thomas H. Dietz

Abstract: Nine species of unionids cleared laboratory-raised *Escherichia coli* from artificial pond water. The six unionid species collected from rivers had higher clearance rates than the three species collected from ponds, when clearance was normalized to millilitres per gram of dry tissue mass per minute. Analysis of variance indicated that all lotic unionids examined form a group with similar clearance rates. When normalized on the basis of gill surface area, rates of clearance by all of the lotic unionids become remarkably similar to one another regardless of mass, but differ significantly from those of the lentic unionids. The cirri found on the laterofrontal cells of the gills of lotic unionids tend to be complex, containing >25 cilia per cirral plate, while the cirri of the unionid species collected from ponds have smaller cirri (<16 cilia per cirral plate). There was a strong correlation between cirral surface area (mm^2) per milligram of dry tissue and clearance rate among the unionid species studied. As a comparison, *Corbicula fluminea* and *Dreissena polymorpha* were also examined and both tended to clear bacteria more rapidly than the lotic unionids.

Résumé : Neuf espèces d'Unionidae ont absorbé tous les *Escherichia coli* élevés en laboratoire dans l'eau d'un étang artificiel. Les six espèces récoltées dans des rivières avaient des taux de clearance plus rapides que les trois espèces provenant des étangs après normalisation de la clearance pour l'exprimer en millilitres par gramme de tissu sec par minute. Les résultats d'une analyse de variance indiquent que tous les unionidés lotiques examinés forment un groupe dont les taux de clearance sont semblables. En normalisant les données en fonction de la surface des branchies, les taux de clearance de tous les unionidés lotiques deviennent remarquablement semblables les uns aux autres, quelle que soit la masse, mais diffèrent significativement de ceux des unionidés lénitiques. Les cirres observés sur les cellules latéro-frontales des branchies chez les unionidés lotiques tendent à être complexes, contenant >25 cils par plaque, alors que les espèces provenant des étangs ont des cirres plus petits (>16 cils par plaque). Il y a une forte corrélation entre la surface du cirre (mm^2) par milligramme de tissu sec et le taux de clearance chez les espèces étudiées. Pour fins de comparaison, *Corbicula fluminea* et *Dreissena polymorpha* ont également été étudiés et les deux espèces ont des taux de clearance de bactéries plus rapides que les unionidés lotiques.
[Traduit par la Rédaction]

Introduction

The relative importance of bacteria as a nutrient used by various suspension-feeding bivalves has been investigated in many different species (Prieur et al. 1990). ZoBell and Feltham (1938) suggested that bacteria were a suitable food source for the marine bivalve *Mytilus californianus*. Stuart et al. (1982) calculated that bacteria in coastal marine environments could not serve as the primary or sole source of nutrients, based on the total amount of carbon present and the relatively inefficient uptake of small bacteria by suspension-feeding bivalves. However, laboratory studies indicate that bacteria in the 1-μm size range can be cleared and assimilated by *Mytilus edulis* and that bacterial clearance is faster in the presence of algae (McHenery and Birbeck 1985). *Mytilus edulis* and several other bivalve species can reduce coliform numbers in both experimental laboratory systems and contaminated waters (Heffernan and Cabelli 1970; Bernard 1989).

For bacteria to be used as a food source, the bivalve species must have the ability to capture small particles and select them as acceptable for transfer to the gut. Owen and McCrae (1976) suggested that this capture of small particles by suspension-feeding bivalves was related to the structure of laterofrontal cirri found in the bivalve species. They noted that species with relatively large cirri clear small particles better than species with small cirri. Jørgensen et al. (1984) obtained similar results comparing *Dreissena polymorpha* with two unionid species. McHenery and Birbeck (1985) also observed differences in rates of clearance of bacteria among three marine bivalve species. They demonstrated that *Chlamys opercularis*, a species with no cirri, had little or no ability to clear bacteria-sized particles.

Silverman et al. (1995) have recently demonstrated that

Received February 26, 1997. Accepted May 8, 1997.

H. Silverman,[1] **J.S. Cherry, J.W. Lynn, and T.H. Dietz.** Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803, U.S.A.
S.J. Nichols. U.S. Geological Survey—Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, U.S.A.
E.C. Achberger. Department of Microbiology, Louisiana State University, Baton Rouge, LA 70803, U.S.A.

[1] Author to whom all correspondence should be addressed (e-mail: cxsilv@unix1.sncc.lsu.edu).

D. polymorpha clears laboratory-cultured *Escherichia coli* 100 times faster than the unionid *Toxolasma* (*Carunculina*) *texasensis* on a dry tissue mass basis. *Toxolasma texasensis* has small cirri and contains less gill surface area per gram of dry mass of tissue than does *D. polymorpha*. Others have previously reported the ability of *D. polymorpha* to clear small particles from the water column in laboratory experiments (Morton 1971; Walz 1978; Sprung and Rose 1988). Cotner et al. (1995) have demonstrated that *D. polymorpha* shows substantial clearance of 0.4- to 1-μm bacteria in field studies conducted in Saginaw Bay, Lake Huron. Differences in the ability of freshwater bivalve species to clear bacteria may give some species greater access to alternative food sources (Jørgensen et al. 1984). However, Lei et al. (1996) reasoned that *D. polymorpha* is not unique and that all freshwater bivalves have the capacity to filter bacteria and other small particles. Silverman et al. (1995) reported that three freshwater bivalve species have different rates of clearance of laboratory-cultured *E. coli* in ^{35}S-labeling studies. However, the assimilation of ^{35}S-labeled amino acids from the bacterial proteins into bivalve proteins normalized to the ^{35}S removed from the bath by mussels was similar (25–30%) for a corbiculid, a unionid, and a dreissenid, regardless of the number of bacteria ingested.

Given the relatively low rate of bacterial clearance reported for some unionids (Jørgensen et al. 1984; Silverman et al. 1995), the relatively higher rates reported for other freshwater unionids (Vanderploeg et al. 1995; Kryger and Riisgård 1988), and the variation in cirral structure known to exist among bivalve species, the current study examines the ability of nine different unionid species to clear laboratory-cultured *E. coli* under controlled laboratory conditions. Comparisons are also made with *Corbicula fluminea* and to *D. polymorpha*. We report that all unionids taken from a river environment had the largest cirri among the unionids examined, and a greater ability to clear bacteria than unionids taken from a pond environment.

Materials and methods

Animals

Toxolasma (*Carunculina*) *texasensis*, *Ligumia subrostrata*, and *Utterbackia* (*Anodonta*) *imbecilis* were collected under permit from a lentic habitat in Baton Rouge, Louisiana. These species were the three most common unionids in a phytoplankton-based pond occupying several hectares. Four unionid species, *Elliptio dilatata*, *Cyclonaias tuberculata*, *Lampsilis ovata*, and *Ptychobranchus fasciolaris*, were collected under permit from the Huron River near Dexter, Michigan; *E. dilatata* was also collected from the Chippewa River near Mount Pleasant, Michigan; *Fusconaia flava* and *Villosa lienosa* were collected near Kisatchie National Forest in central Louisiana. All of these animals are from medium- to fast-running (1–2 m/s) second-order rivers that are oligotrophic and are referred to as lotic species. *Corbicula fluminea* were collected from the Tangipahoa River, Louisiana. *Dreissena polymorpha* were collected from Lake Erie at the mouth of the Raisin River in Monroe, Michigan, and from the Mississippi River near Baton Rouge. All species were kept under laboratory lighting conditions (22 ± 2°C) in aerated artificial pond water (APW; in mM, 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, 0.05 KCl); for *D. polymorpha*, the APW also contained 0.2 mM Mg_2SO_4 (Dietz et al. 1994). Animals were maintained unfed in the laboratory for at least 5 days before use.

Labeling of bacteria

Escherichia coli JM83 (Messing 1979) was used for all experiments. The conditions for ^{35}S labeling have been described previously (Silverman et al. 1995). The growth medium for bacterial culture consisted of 5 g glucose, 810 mg NH_4Cl, and 82 mg $MgCl_2 \cdot 7H_2O$ per litre of 0.05 M potassium phosphate buffer, pH 7.2. Five millilitres of a trace salts solution ($CaCl_2 \cdot 2H_20$, 2 g; $MnSO_4 \cdot H_2O$, 1 g; and $FeSO_4 \cdot 7H_2O$, 0.5 g dissolved in 1 L of 0.1 M HCl) was added to the growth medium. In addition, 20 μg/mL L-proline and 5 μg/mL thiamine are required for *E. coli* JM83 growth. The carbon source, trace salts solution, L-proline, and thiamine were sterilized separately from the rest of the medium. *Escherichia coli* were labeled with ^{35}S by growing the bacteria at 37°C with shaking aeration for at least four generations in the growth medium containing 5 μCi/mL carrier-free $Na_2{}^{35}SO_4$ (DuPont, NEN; 1 Ci = 37 GBq). Incorporation of ^{35}S during growth of *E. coli* was measured with a liquid scintillation counter. The bacteria were precipitated in 10% trichloroacetic acid and collected on a No. 25 glass-fiber filter (Schleicher and Schuell). Greater than 70% incorporation of the radiolabel into *E. coli* protein was routine.

The final cell density of cultures was approximately $1.3–2 \times 10^9$ bacteria/mL. Labeled bacteria were collected by centrifugation, washed once in the growth medium without carbon source, and stored in APW at a concentration of 3×10^9 bacteria/mL. The concentration of bacteria was determined by direct microscopic count using a hemocytometer. The cells were stored on ice in pond water until use. Cell dimensions of labeled bacteria were measured from photomicrographs. *Escherichia coli* grown in this medium were 2.3 ± 0.1 μm long and 0.9 ± 0.0 μm wide (mean ± SE; $N = 50$) and did not clump.

Feeding experiments with *E. coli*

All feeding experiments were carried out in individual chambers aerated for the duration of the experiment. The size of the experimental chambers used was adjusted according to the size of the species to be tested. The volume in the chamber was chosen to cover the animal placed with the siphons positioned towards the surface of the container (20–200 mL). This served to reduce the amount of radioactive waste produced. Disappearance of bacterial radiolabel followed first-order kinetics in these experiments for all species tested. The experiment was started by the addition of bacteria as soon as the bivalves began siphoning. Siphoning typically began within 10 min of the animals being placed in the experimental chamber containing pond water.

Each chamber contained $1–2 \times 10^7$ bacteria/mL representing about 1.2×10^5 dpm ^{35}S/mL. Controls for bacterial degradation and loss of ^{35}S to the water column were as previously described (Silverman et al. 1995). Each day the *E. coli* stock solution (in APW at 4°C) was centrifuged and resuspended in APW. The discarded supernatant was assayed for ^{35}S. Using this assay procedure, we found that *E. coli* did not deteriorate and that all ^{35}S (>99%) added to an experimental tube was associated with intact bacteria in the APW and not with breakdown products in the supernatant. Control chambers without bivalves received labeled bacteria, were aerated for 20 min, and analyzed. The bath solutions from these tubes were passed through a Millipore filter (0.22 μm) to trap the bacteria. Virtually all of the label in each tube (>99.5%) was on the filter, while the supernatant contained less than 1500 dpm/mL. Thus, <0.5% of the radioactivity was in the nonparticulate material of the assay medium.

Measurement of bivalve clearance was initiated by collecting a sample (t_0) of the bath shortly after inoculation of the pond water with bacteria. Bath water samples (100 μL) were taken at 0, 30, 60, and 90 min for unionids and at 5- to 10-min intervals for *C. fluminea* and *D. polymorpha*. For all samples, ^{35}S radioactivity

Table 1. Rates of uptake of ^{35}S-labeled *E. coli* by unionid bivalves.

	N	Dry mass (g)	Rate constant (per minute)	Clearance	
				mL · g^{-1} · min^{-1}	μL · mm^{-2} · min^{-1}
Lentic unionids					
Utterbackia imbecilis	9	1.203±0.181	−0.008±0.001	0.60±0.17*a*	0.19±0.05*a*
Toxolasma texasensis	19	0.454±0.087	−0.005±0.001	0.63±0.08*a*	0.37±0.05*a*
Ligumia subrostrata	10	1.997±0.813	−0.016±0.001	1.02±0.21*a*	0.34±0.07*a*
Lotic unionids					
Fusconaia flava	3	1.195±0.033	−0.119±0.012	4.99±0.46*b*	2.00±0.18*b*
Lampsilis ovata	3	1.180±0.126	−0.091±0.023	5.90±1.44*b*	3.21±0.79*b*
Villosa lienosa	3	0.913±0.070	−0.082±0.022	6.56±1.30*b*	2.01±0.40*b*
Elliptio dilatata	16	1.080±0.178	−0.059±0.007	7.66±0.99*b*	3.76±0.48*b*
Ptychobranchus fasciolaris	5	1.870±0.401	−0.085±0.016	8.06±1.73*b*	3.03±0.65*b*
Cyclonaias tuberculata	3	0.670±0.073	−0.134±0.004	19.17±2.04*b*	4.60±0.49*b*

Note: Clearance rates (mean ± SE) are normalized to either grams dry tissue or square millimetres of gill surface area. Clearance rates within each column that are significantly different at $p < 0.05$, using ANOVA and Tukey–Kramer comparisons, are followed by different letters.

was determined with a liquid scintillation counter (Wiegman et al. 1975).

Cirral structure

Differences in cirral structure and distribution were examined and described using scanning electron microscopy. Tissue fixation followed modification of previously described procedures (Richard et al. 1991; Silverman et al. 1996). Gills from 3–10 specimens per species were excised from the animal and photographed. Each gill was placed flat (as a demibranch pair) on a piece of 35-mm photographic film and immediately frozen in vapor at the surface of liquid N_2. Cold 2% glutaraldehyde in phosphate buffer (pH 7.6) was poured directly over the frozen gill. Prior to dissection, a blood sample from each species was taken by cardiac puncture (Fyhn and Costlow 1975) and the osmolality determined (Precision Systems Osmette). The phosphate buffer was 35–60 mosmol as appropriate to match blood osmolality of the bivalve species. Following the 1-h fixation in glutaraldehyde, the gill was rinsed in buffer and post-fixed in 1% osmium tetroxide. Gills were dehydrated in an ethanol series, critical-point dried, mounted on aluminum stubs using carbon tape, and sputter-coated with gold–palladium (20 nm). Immediately following the critical-point drying procedure, demibranch pairs were rephotographed. Gills were examined using a Cambridge 260 scanning electron microscope. For calculations of cirral size and gill surface areas it was necessary to measure the shrinkage associated with tissue processing for scanning electron microscopic examination. Shrinkage of each gill was determined from photographs of the demibranch pairs using Image 1/AT morphometric analysis (Silverman et al. 1995). Shrinkage of the total gill area and along the length (length of filament or dorsal–ventral axis) and width of the gill (anterior–posterior axis) was determined by comparing demibranchs photographed before fixation with photographs taken after critical-point drying. Filament dimensions, number of cirri per filament, and gill area were determined from scanning electron micrographs.

Determination of the number of cilia per cirral plate often is difficult because the cirri either tend to split during preparation or can be obscured if they are examined within the filamental structure. This is a problem, particularly for species with short cirri. Therefore, cilia making up the body of a cirrus in this study were always enumerated by examining filaments where cirri had been detached from the cell or where laterofrontal cirral cells were isolated from the filament.

Calculations and statistical analysis

Disappearance of radioactive label from the bath was exponential and followed first-order rate kinetics (rate constant = $\ln(D_0/D_t) \cdot t^{-1}$; D was the disintegration rate (dpm/mL) at times 0 and t). The bacterial clearance rate (C; mL · min^{-1}) for each mussel was calculated using the equation $C = V/t \cdot \ln(D_0/D_t)$, where V is the volume (mL) and D_0 and D_t are the bacterial radiolabel concentrations defined above (see Riisgård 1988). Data are reported as mass-specific clearance (dry soft-tissue mass) and expressed in millilitres per gram of dry tissue per minute. The clearance rate based on gill surface area was also calculated and expressed in microlitres per square millimetre of gill surface area per minute, using gill measurements as described above.

Cirral surface area is related to the number of ciliary pairs composing a cirrus. The average width of a cirrus was approximately 2 μm. Thus, our average calculated area per cirrus was 4.3×10^{-7} mm^2 per ciliary pair as determined from 20 representative specimens of all species. The cirral surface area (CSA), expressed in square millimetres per milligram of dry tissue, was calculated using the equation CSA = (number of cilia per cirri) (number of cirri per milligram of dry tissue) (4.3×10^{-7} mm^2/cilium).

Data are expressed as mean ± standard error (SE) and differences between species or treatments were determined by analysis of variance (ANOVA) and considered significant if $p < 0.05$. Tukey–Kramer post-ANOVA tests were used to determine differences between average values among the bivalve species. ANCOVA was used to analyze covariance between species and mass, with clearance as the dependent variable. Differences between regressions were determined by Student's t test and considered significant if $p < 0.05$.

Results

Clearance of ^{35}S-labeled bacteria by unionids

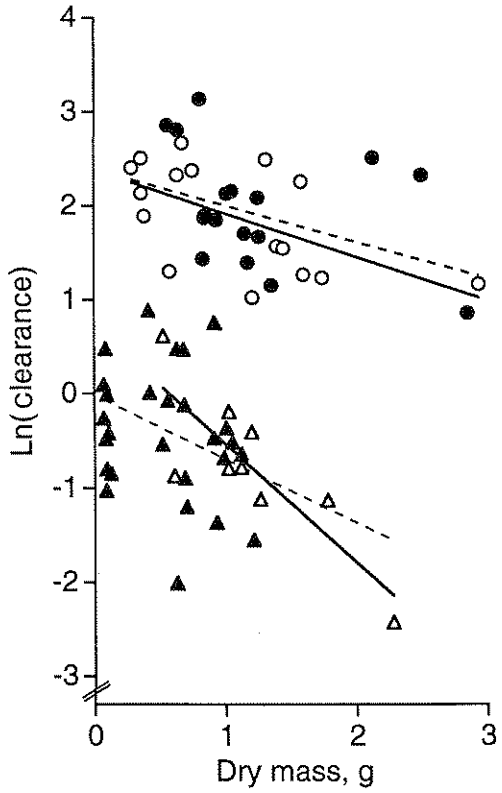
Rates of clearance of *E. coli* from the experimental chambers for each of the unionid species are shown in Table 1. The unionids collected from ponds had the slowest clearance

Table 2. Morphometric characteristics of gills from representative unionid bivalves used in bacterial clearance studies.

Bivalve species	Live gill area ($mm^2 \cdot mg^{-1}$ dry tissue)	No. of cilia/cirrus	No. of cirri/ mg dry tissue	No. of cirri/ mm^2 live gill	Cirral area (mm^2)/ mg dry tissue
		Lentic unionids			
Utterbackia imbecilis	3.13	14–15	33 877	10 828	0.203
Toxolasma texasensis	1.66	11–13	19 919	16 360	0.191
Ligumia subrostrata	3.01	12–16	48 908	16 233	0.252
		Lotic unionids			
Elliptio dilatata	2.04	30–32	32 750	16 025	0.422
Lampsilis ovata	1.84	26–35	40 568	22 059	0.454
Ptychobranchus fasciolaris	2.66	25–32	44 764	16 838	0.481
Fusconaia flava	2.49	25–30	46 313	18 572	0.498
Villosa lienosa	3.27	25–30	55 493	16 988	0.597
Cyclonaias tuberculata	4.17	38–42	70 264	16 851	1.148

Note: Morphometric values are the average or range for 3–10 specimens of each species.

Fig. 1. Dry tissue mass versus the natural logarithm of mass-specific rates of clearance of *E. coli* by *Utterbackia imbecilis* (Δ, solid line), $r = 0.835$, $p < 0.01$, $N = 9$; *Elliptio dilatata* (○, solid line), $r = 0.574$, $p < 0.05$, $N = 16$; all lotic species of unionids examined (●, broken line), $r = 0.446$, $p < 0.01$, $N = 37$; and all lentic species (▲, broken line), $r = 0.442$, $p < 0.05$, $N = 33$.



rates, 0.6 to about 1 $mL \cdot g^{-1}$ dry tissue $\cdot$ min^{-1}, while lotic bivalves cleared bacteria at a rate of 5–19 $mL \cdot g^{-1}$ dry tissue $\cdot$ min^{-1}. ANOVA followed by Tukey–Kramer comparisons indicated that all lotic unionid species fell into a group which cleared bacteria at a significantly greater rate than the lentic unionids (Table 1).

Table 3. Regression coefficients of mass-specific rates of clearance of *E. coli* by the freshwater bivalves presented in Fig. 2.

	N	Slope	Intercept	*F*
Dreissena polymorpha	38	$-33.38 \pm 5.26a$	$5.15 \pm 0.18a$	40.25
Corbicula fluminea	63	$-7.18 \pm 0.71b$	$4.41 \pm 0.20b$	103.52
Utterbackia imbecilis	9	$-1.26 \pm 0.31c$	$0.73 \pm 0.41d$	16.08
Elliptio dilatata	16	$-0.46 \pm 0.18d$	$2.39 \pm 0.23c$	6.87

Note: Comparisons of the slopes and intercepts (mean ± SE) for all species indicate that they are all significantly different ($p < 0.01$). The ANOVA *F* statistics for the regression lines are all significantly different ($p < 0.01$).

Clearance rates are routinely standardized on the basis of animal dry tissue mass. However, particle-capture ability is related to gill surface area, and structural variations in the gill have a significant impact. Therefore, clearance rates are expressed on a gill surface area basis (Table 1). The surface area calculations based on morphometric analysis of the gill structure of the freshwater mussels are presented in Table 2. When expressed on this basis, the clearance rates for the lentic unionids ranged from 0.2 to 0.4 $\mu L \cdot mm^{-2}$ gill $\cdot$ min^{-1}, while values for the lotic species were 2.0–4.6 $\mu L \cdot mm^{-2}$ gill $\cdot$ min^{-1} (Table 1). ANOVA indicated that the lotic and lentic unionids separate into two significantly different groups.

We also examined the relationship between clearance rate and mass within species where the range in mass allowed allometric regression analysis. Because of our concern for unionid distribution and the current stress on many of their populations, we minimized the number of unionids used in this study. In an attempt to derive information on a mass basis for the two groups described above, we chose one abundant representative species from each group: *U. imbecilis* for the lentic animals and *E. dilatata* for the lotic animals. There was at least a threefold range in mass, and the relationship between mass and clearance rate is shown in Fig. 1. Both species showed significant regressions relating the natural logarithm of mass-specific clearance rate to dry tissue mass. The intercept was 2.39 for the lotic species and 0.73 for the lentic unionids, representing 10.9 and 2.1 $mL \cdot g^{-1}$

Fig. 2. Dry tissue mass versus the natural logarithm of mass-specific rates of clearance of *E. coli* by *Dreissena polymorpha* (□), $r = 0.727$, $p < 0.01$, $N = 38$, and *Corbicula fluminea* (♦), $r = 0.793$, $p < 0.01$, $N = 63$. For comparative purposes, *Utterbackia imbecilis* (△) representing the lentic unionid species and *Elliptio dilatata* (○) representing the lotic unionid species from Fig. 1 are also shown.

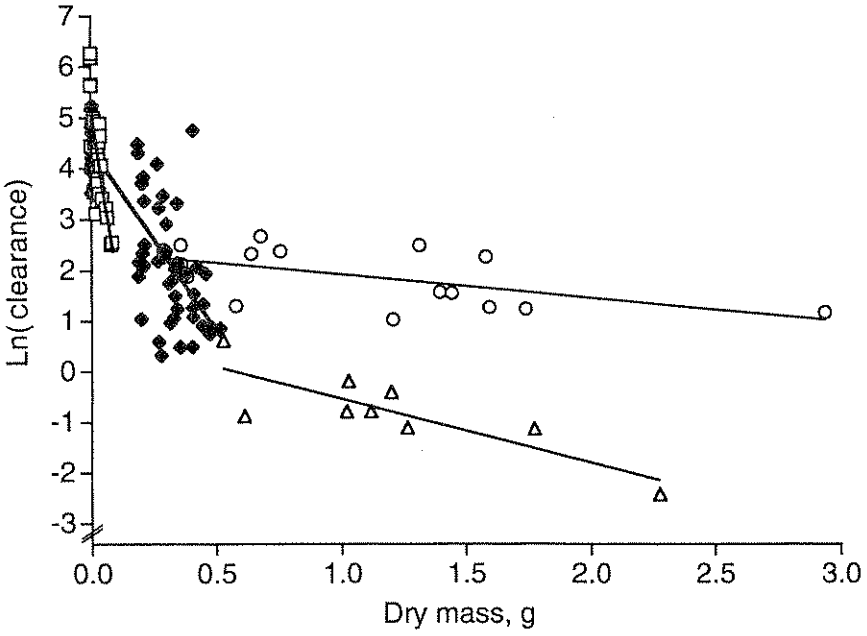


Table 4. Rates of uptake of ^{35}S-labeled *E. coli* by the freshwater bivalves *Corbicula fluminea* and *Dreissena polymorpha*.

		Dry mass	Rate constant	Clearance	
	N	(g)	(per min)	mL · g^{-1} · min^{-1}	μL · mm^{-2} · min^{-1}
Corbicula fluminea	52	0.2225±0.0244	−0.067±0.007	36.71±6.95*a*	25.50±4.83*a*
Dreissena polymorpha	38	0.0278±0.0031	−0.082±0.009	102.78±18.59*b*	7.73±1.40*b*

Note: Clearance rates (mean ± SE) within a column followed by a different letter are significantly different at $p < 0.05$, using ANOVA folowed by Tukey–Kramer comparisons.

dry tissue · min^{-1}, respectively (Table 3). The intercepts differ significantly and indicate the theoretical maximum clearance rate for the smallest specimens of a species. The slopes of the regressions were shallow and not significantly different (Fig. 1). ANCOVA results indicated that the regressions comparing the lentic with the lotic species were significantly different from one another. Because the ANOVA indicated that all species from the same habitat were similar, we pooled the data from all lotic or all lentic unionids and calculated pooled regressions (Fig. 1). The ANCOVA results indicated no difference between the pooled regressions and the regressions for the representative species within each group. However, the differences between lentic and lotic unionid species persisted whether the data were pooled or analyzed using the two representative species.

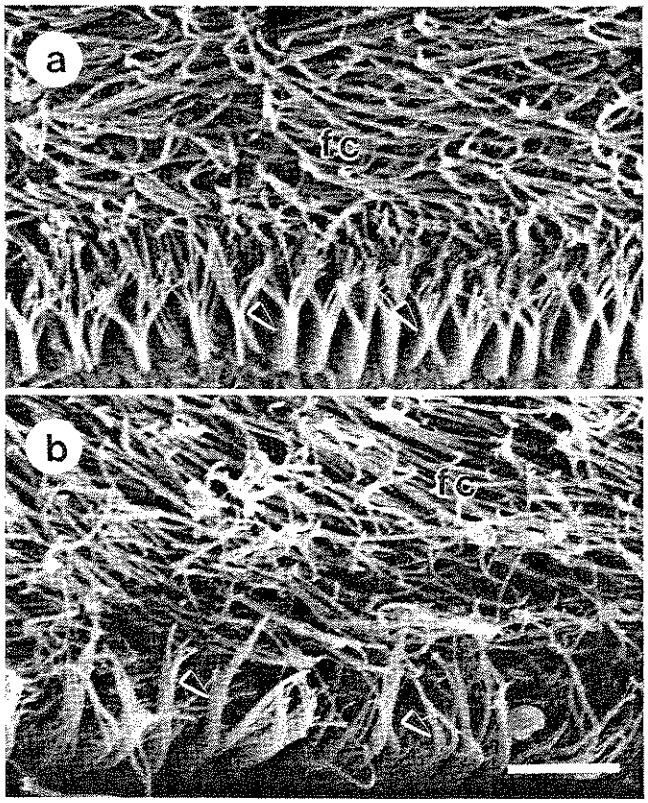
Clearance of ^{35}S-labeled bacteria by *D. polymorpha* and *C. fluminea*

Rates of clearance of bacteria by the two non-indigenous species, *D. polymorpha* and *C. fluminea*, are presented (Table 4) and compared with those of the representative unionid species in Fig. 2. The slopes of the relationship between mass and clearance rate for the dreissenid and the corbiculid (Table 3) were steeper than those found for the representative lentic and lotic unionid species. While ANCOVA may not be strictly correct, owing to the minimal overlap in mass between species, the use of mass as a means of standardizing clearance rates among animals allowed comparison with other studies on bivalve suspension-feeding. An ANCOVA comparison of all four species indicated significant differences among them and that the mass–species interactions are highly significant (Fig. 2).

Variation in cirral structure

The unionids from ponds that we examined all had small cirri and few cilia per cirral plate (Figs. 3, 4, and 5, Table 2) compared with the other bivalves in this study. There were 16 or fewer cilia per cirral plate in lentic unionid species (Figs. 4*a*, 5*b*, and 5*d*). All the unionid species taken from rivers had 25 or more cilia per cirral plate (Table 2, Figs. 4*b*, 5*a*, and 5*c*). In comparison, *D. polymorpha* and *C. fluminea* have a complex cirrus consisting of 32–42 cilia per cirral plate (Silverman et al. 1995). While the numbers of cilia per cirral plate differ between lentic and lotic unionids, the numbers of cirri per square millimetre of gill were not significantly different. However, the total surface area of the cirri present

Fig. 3. Scanning electron micrographs of the lotic unionid *Ptychobranchus fasciolaris* (*a*) and the lentic unionid *Toxolasma texasensis* (*b*). The laterofrontal cirri (arrowheads) and the frontal cilia (*fc*) of one filament are identified. The cirri are smaller and somewhat less organized in appearance in *T. texasensis* than in *P. fasciolaris*. The cirral tips are longer and cover more of the frontal surface in *P. fasciolaris* than in *T. texasensis*. Scale bar = 5 μm.

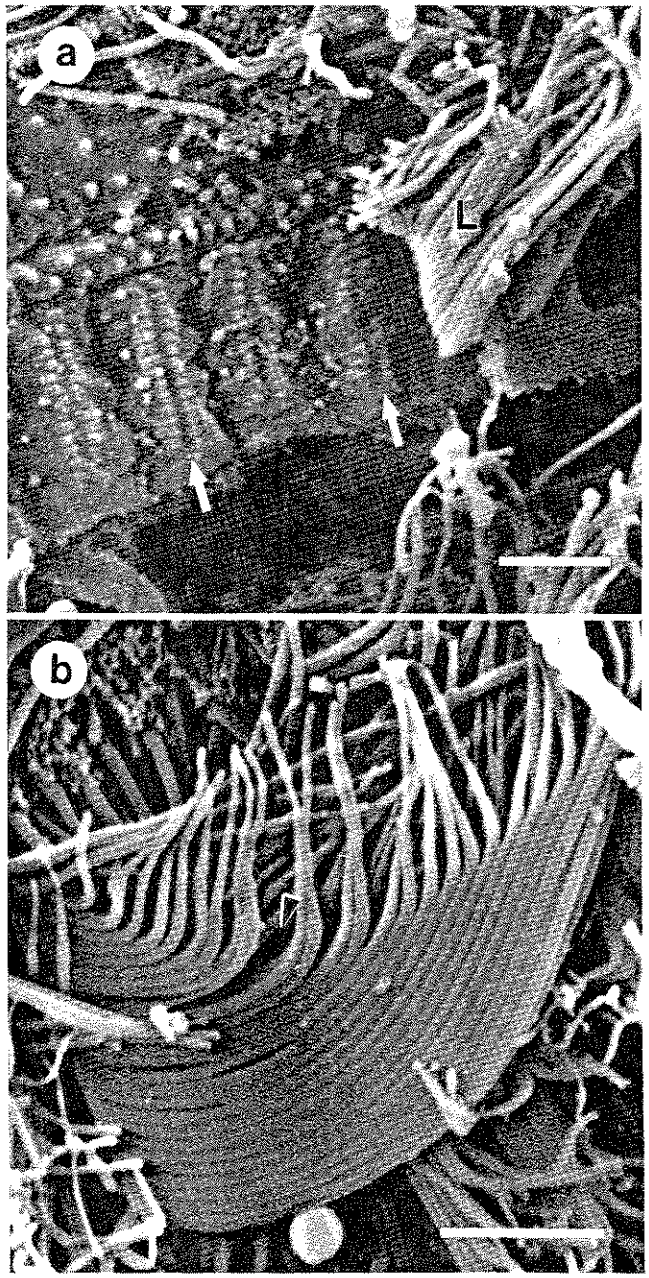


on the gill filaments will be a function of the number of cilia composing the cirrus. The cirral surface area per milligram of dry tissue was calculated for each of the unionid species (Table 2). All lotic species had more cirral surface area per milligram of dry tissue than the lentic species. The relationship between bacterial clearance rate and cirral surface area for each of the unionid species is shown in Fig. 6. The data fit a power function when the nine unionid and the two non-indigenous species (*Dreissena* and *Corbicula*) are pooled ($r = 0.85$, df = 9, $p < 0.01$). If the unionid species only are considered, the correlation between cirral surface area and clearance rate is even stronger ($r = 0.96$, df = 7, $p < 0.01$).

Discussion

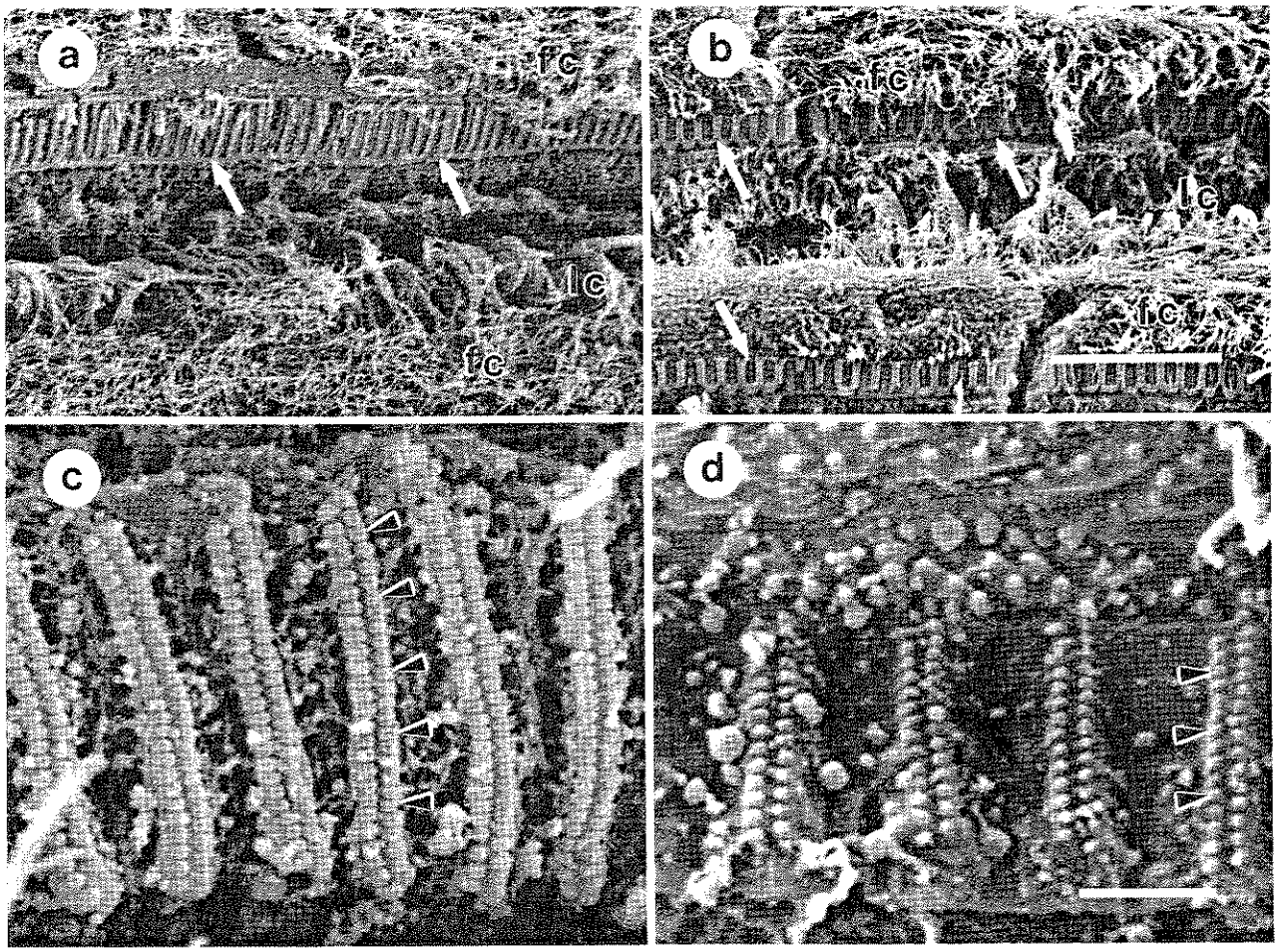
Lotic unionid bivalves clear laboratory-raised *E. coli* at a significantly higher rate than lentic unionids under the experimental conditions described here. This difference in clearance rates was found whether the rate was expressed in millilitres per gram dry mass per minute or in microlitres per square millimetre of gill area per minute. Using either expression of clearance rate, the difference between the bivalve groups correlates well with differences in cirral structure between unionids from pond and river habitats.

Fig. 4. Scanning electron micrographs of gills with some cirri detached from the laterofrontal cells. (*a*) A laterofrontal cirrus (L) from *Ligumia subrostrata*, a lentic unionid. This cirrus is partially pulled free at its base. The attachment sites (arrows) of several other cirri from which the cirral cilia have been detached are visible. These sites facilitate determination of the number of cilia per cirrus in any particular species. (*b*) A laterofrontal cirrus from *Lampsilis ovata*, a lotic unionid. A small split (arrowhead) has formed in this rather large cirrus. Such splits are common and cause difficulty in counting cirral cilia in intact gill preparations. Scale bars = 2 μm.



Unionids from the lotic habitat have clearance values of 2–4.6 $\mu L \cdot mm^{-2}$ gill $\cdot min^{-1}$, while lentic unionids have values 6–20 times lower. All of the lentic unionids examined have small laterofrontal cirri compared with the lotic bivalves (Table 2).

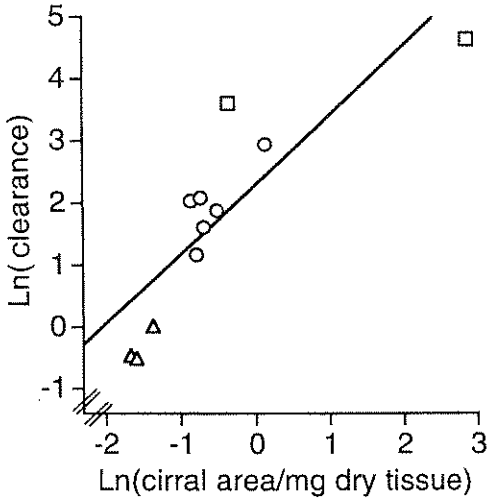
Fig. 5. Comparison of gills from the lotic unionid *Ptychobranchus fasciolaris* with those from the lentic unionid *Ligumia subrostrata*. These images allow the accurate counting of cilia per cirral plate. Parts of two filaments from *P. fasciolaris* (*a*) and *Ligumia subrostrata* (*b*) are shown at the same magnification. The cirri in the topmost filament have been detached, leaving a "railroad track" (arrows) appearance formed by the apical surface of the laterofrontal cells. Despite the disruption of the tissue the remaining frontal cilia (*fc*) and cirri (*lc*) can be seen associated with the filaments. The width of the cirral "track" in *L. subrostrata* (lentic) is much smaller than that seen for *P. fasciolaris* (lotic). At higher magnification, *a* to *c* and *b* to *d*, respectively, is a nearly vertical view of the apical surface of laterofrontal cirral cells showing regions of sheared-off cilia. The previous location of each cilium is visible as a knob (arrowheads). Scale bars: 20 μm for *a* and *b* and 2 μm for *c* and *d*.



The laboratory-grown *E. coli* used in these studies are rather large, 2.3 μm long and 0.9 μm wide. Lei et al. (1996) have recently suggested that all freshwater species can filter particles of this size effectively, based on the ability of *D. polymorpha* to clear microspheres and compared with selected clearance values reported in the literature. Factors likely to influence the ability of freshwater mussels to filter bacteria-sized particles are the gill surface area (Kryger and Riisgård 1988; Silverman et al. 1995; Lei et al. 1996), the microanatomy of the gill filaments, and the particle-capture mechanics of the gill (Owen and McCrae 1976; Silverman et al. 1996).

The present study demonstrates that while each of the freshwater species examined can capture bacteria, clearance rates vary among bivalve species depending, at least in part, on cirral structure. There is a striking similarity in filter-feeding across the many suspension-feeding bivalve families (Jørgensen 1990; Lei et al. 1996). Thus, generalized mechanisms of suspension-feeding in bivalves must be considered. In a series of papers, Beninger and his co-workers have described the process of suspension-feeding in a number of marine bivalve species as requiring at least three components: (1) particle capture, (2) transport, and (3) selection (Beninger et al. 1992, 1993; Ward et al. 1993). For particle capture, Lei et al. (1996) stress that all freshwater bivalve species have similarly structured gills, therefore the general mechanism of particle capture is likely to be the same. The variation will come from the structural differences of the gills of different species, particularly in the size of the gill, and perhaps in regulation of gill function. Among the bivalves examined in this study, mass (or size) was important, being inversely related to the clearance rate expressed in millilitres per gram dry tissue per minute. However, other factors must be more important to account for the differences in clearance rate between similar-sized unionids (compare the two representative unionids in Fig. 7). Gill sizes in the two unionid groups were similar, ranging from 1.66 to 3.13 mm^2 gill · mg^{-1} dry tissue in the lentic unionids and

Fig. 6. Relationship between the natural logarithms of rates of clearance of bacteria (mL · g^{-1} dry tissue · min^{-1}) and cirral area among the freshwater bivalves. △, lentic unionids; ○, lotic unionids; □, non-indigenous exotic species.

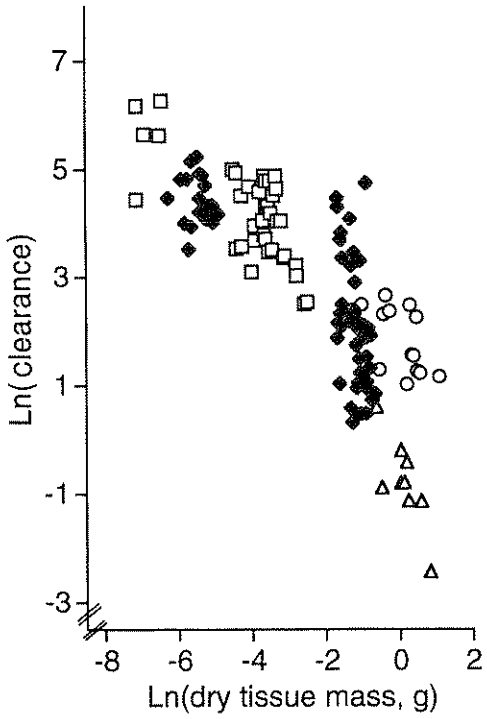


1.84−4.17 mm^2 gill · mg^{-1} dry tissue in the lotic bivalves, group averages being 2.6 and 2.7 mm^2 gill · mg^{-1} dry tissue, respectively. Although *U. imbecilis* tends to have a larger gill surface area than similar-sized *E. dilatata*, it has significantly lower clearance rates (Table 2, Figs. 6 and 7). This indicates that gill surface area alone is not the basis for the differences in the rates of clearance of *E. coli* between the lentic and lotic unionids.

In contrast, the differences in rates of clearance of *E. coli* among the nine unionid species studied here correlate well with both the structure of the gill cirri and the collection sites (i.e., lentic versus lotic habitats). Bivalves obtained from river environments had the greatest number of cilia forming a cirrus. All of the lotic unionids had cirri composed of 25−35 cilia per cirral plate, while lentic unionids had cirri containing <16 cilia. Indeed, calculated cirral area per gram dry tissue correlates well with the bacterial clearance rates for the unionid species studied here (Fig. 6). The lotic bivalves were collected from river systems, where the amount of algae and phytoplankton would ordinarily be substantially less than that found in the ponds where the lentic unionid species were collected.

The experiments described here were all laboratory based and did not test the ability of these animals to select prey particles from mixed seston. Bayne et al. (1993) have documented that in several marine species, clearance rates of specific particulates are related to the seston load. The clearance rates reported here occurred under highly defined laboratory conditions and do not take into account seston effects, temperature effects, particle load or other environmental variables. We controlled most of the potential variables and used a well-defined bacterial assay system (Silverman et al. 1995). Thus, the data may not represent the clearance rates that these various species would show in their native environment. These data do represent the relative differences between unionid species in their ability to clear bacteria. By comparison, we conducted a preliminary study of algal clearance rates in five of these unionid species and found that all species cleared a mixed culture (10^5 cells/mL)

Fig. 7. Plot of ln(mass-specific clearance) versus ln(dry mass) for *Dreissena polymorpha* (□), *Corbicula fluminea* (♦), *Elliptio dilatata* (○), and *Utterbackia imbecilis* (△).



of *Chlorella* sp. (4−8 μm), *Chlamydomonas* sp. (7−10 μm), and *Synodessmus* sp. (14 × 3 μm) at a rate of 10−23 mL · g^{-1} dry tissue · min^{-1}. Interestingly, *U. imbecilis*, a lentic unionid, cleared the algae at the same rate as four different lotic unionid species. Thus, cirral structure is apparently not as important in explaining differences in algal clearance rates among unionids.

Lei et al. (1996) indicated that the size of the gill is the major determinant of clearance ability within a species, and speculate that this relationship will hold across freshwater species. Our data confirm that the size of the gill is an important component in a bivalve's ability to clear particles, but it is not the only determinant, and the relationship between gill size and clearance rate varies markedly between species.

In this study, larger size (mass) was associated with lower mass-specific clearance rates. For the unionids, the slope of this relationship tended to be rather shallow, in part because of the constancy of the ratio of gill surface area to dry tissue mass among the unionids. Indeed, when clearance is expressed on a gill surface area basis, the small variation between mass-specific clearance rates among the unionid species is reduced, as all of these species show a strong relationship between clearance rate and gill surface area. The smaller *D. polymorpha*, and to a lesser extent *C. fluminea*, show a much steeper slope in mass-specific clearance rates (Fig. 2), with *D. polymorpha* showing the highest rate. However, on a gill surface area basis, the rate of clearance of bacteria by *C. fluminea* is greater than that by *D. polymorpha* and many times higher than that by any of the unionids. The steepness of the slope could relate to a number of factors, including gill water flow, ostial area, musculature, and regulatory capabilities in addition to gill area and cirral structure (Foster-Smith 1975, 1976; Way et al. 1990). The

seeming contradiction is more likely related to the ability of *C. fluminea* to move more water across the gill than *D. polymorpha* than to the differences in surface area of gill per gram dry mass (see Way et al. 1990; Silverman et al. 1995).

Thus, an important factor in the differential ability of freshwater bivalves to use laboratory-cultured bacteria is the structural difference in the gill (cirral surface area). The ability of each of the unionids to clear bacteria was consistently related to its distribution in lentic or lotic environments. The correlation between a more complex cirral structure and the uniformly higher rate of clearance of bacteria-sized particles by all of the lotic unionids than by the lentic unionids is evidence that the more complex cirri enhance the ability to capture small particles (Silverman et al. 1995). It also provides further evidence for the correlation of complex cirri on the gills with the capture of small particles by other bivalves with lamellibranch gills (Atkins 1938; Moore 1971; Owen and McCrae 1976; Silvester and Sleigh 1984; McHenery and Birbeck 1985; Nielsen et al. 1993; Riisgård et al. 1996; Silverman et al. 1996).

Finally, a preliminary report has appeared indicating differential starvation between unionid species infested by *D. polymorpha* (Baker and Hornbach 1996). Ricciardi et al. (1996) also suggest differential mortality among unionid species related to energy-storage demands. Whether such species-specific effects are related to structural differences in the gill remains to be determined. There is evidence that the unionid species in the Mississippi and Ohio rivers have tolerated zebra mussel infestations better than species in Lake Erie and Lake St. Clair (Haag et al. 1993; Hunter and Bailey 1992; Miller and Payne 1996; Payne and Miller 1997).

Payne et al. (1995) have suggested that variation in palp and gill size within species may result from developmental plasticity and that both *D. polymorpha* and *C. fluminea* alter the size of these organs in response to the particle load in their environment. Whether integrated structural components of the gill, such as cirral structure and ostial area, show similar plasticity in response to different environmental stresses or such differences are more genetically fixed remains to be determined.

Acknowledgements

We thank Ron Bouchard for his technical assistance. Microscopic work was carried out in the Louisiana State University Life Sciences Microscopy Facility. Dr. John Scheide, Central Michigan University, collected the *Elliptio dilatata* from the Chippewa River and Dr. Paul Johnson, University of Texas at Arlington, collected the *Fusconaia flava* and *Villosa lienosa* used in this study. The work was supported by Louisiana Sea Grants R/ZM-1-PD and National Sea Grant NA46RG0096 Project R/ZMM-1.

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