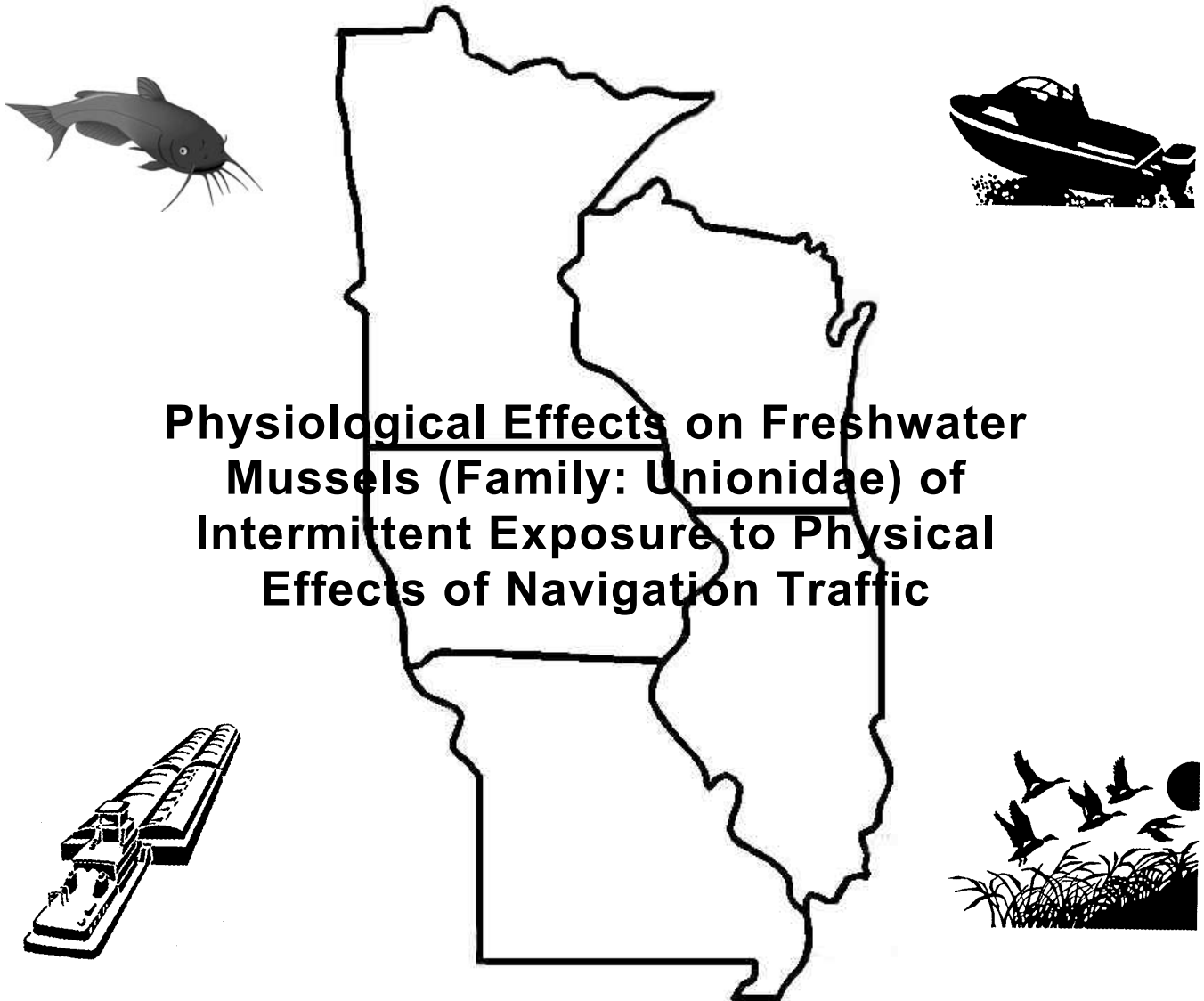


Interim Report For The Upper Mississippi River - Illinois Waterway System Navigation Study



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of Engineers**

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Physiological Effects on Freshwater Mussels (Family: Unionidae) of Intermittent Exposure to Physical Effects of Navigation Traffic

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Preface

The work reported herein was conducted as part of the Upper Mississippi River - Illinois Waterway (UMR-IWW) System Navigation Study. The information generated for this interim effort will be considered as part of the plan formulation process for the System Navigation Study.

The UMR-IWW System Navigation Study is being conducted by the U.S. Army Engineer Districts of Rock Island, St. Louis, and St. Paul under the authority of Section 216 of the Flood Control Act of 1970. Commercial navigation traffic is increasing and, in consideration of existing system lock constraints, will result in traffic delays that will continue to grow in the future. The System Navigation Study scope is to examine the feasibility of navigation improvements to the Upper Mississippi River and Illinois Waterway to reduce delays to commercial navigation traffic. The study will determine the location and appropriate sequencing of potential navigation improvements on the system, prioritizing the improvements for the 50-year planning horizon from 2000 through 2050. The final product of the System Navigation Study will be a Feasibility Report which will be the decision document for processing to Congress.

This investigation was performed under the general supervision of Dr. John W. Keeley, Acting Director, Environmental Laboratory (EL), U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS, and Dr. C. J. Kirby, Chief, Ecological Research Division (ERD), EL, and under the direct supervision of Dr. Alfred F. Cofrancesco, Chief, Aquatic Habitat Group, ERD.

This report was authored by Drs. Barry S. Payne and Andrew C. Miller and Mr. Larry Shaffer, Aquatic Ecology Branch, ERD. The following students assisted with this project: Thomas Ussery, University of Texas at Arlington; William Green, University of Southern Mississippi, Hattisburg, MS; and Stacy Poor, Millsaps College, Clinton, MS.

At the time of publication of this report, Dr. James R. Houston was Director of ERDC, and COL James S. Weller, EN, was Commander.

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1 Introduction

Concern Over the Environmental Effects of Commercial Navigation Traffic

Much has been written on the environmental effects of movement of commercial navigation vessels in the last 10-15 years (Virginia Polytechnic Institute and State University 1975; Academy of Natural Sciences of Philadelphia 1980; Berger Associates, Ltd. 1980; Sparks, Thomas, and Schaeffer 1980; U.S. Army Corps of Engineers 1980; Lubinski et al. 1980, 1981; Environmental Science and Engineering (ESE) 1981, 1988; Kennedy, Harber, and Littlejohn 1982; Rasmussen 1983; Simons et al. 1981; Simons, Ghaboosi, and Chang 1987; Wright 1982; Wuebben, Brown, and Zabilansky 1984; and Nielsen, Sheehan, and Orth 1986). The increasing use of inland waterways to transport bulk commodities (Dietz et al. 1983) and a few recent articles on impacts of waterway use in Europe (Brookes and Hanbury 1990, Haendel and Tittizer 1990) indicate that this issue will remain important well into the 21st century.

Pulses of increased velocity or turbulence, and elevated suspended solids, are major detrimental effects of vessel passage on benthic organisms. Tolerances of many species of aquatic organisms to sustained, specific levels of turbulence, water velocity, or suspended solids are known either from laboratory simulation or field studies. Laboratory studies in which navigation effects were simulated include those on mussels (Aldridge, Payne, and Miller 1987; Payne and Miller 1987) as well as fish eggs (Morgan et al. 1976, Holland 1986), fish larvae (Killgore, Miller, and Conley 1987; Holland 1986; Payne, Killgore, and Miller 1991), and plankton (Stevenson et al. 1986). Results of most studies demonstrated that mortality or physiological stress could be measured under conditions corresponding to high traffic intensity or unusually high levels of turbulence or total suspended solids.

In the field, discharge, flow patterns, bathymetry, and sediment characteristics have complex influences on vessel-induced disturbances. It is difficult to estimate an organismal response to these intermittent physical effects, and it is more difficult to predict long-term responses of natural populations to such disturbances. Results of navigation-related field studies are often characterized by extreme spatial and temporal variability, and clear patterns of navigation effects often cannot be discerned (Sparks, Thomas, and Schaeffer 1980; Bhowmik et al. 1981a, b; Seagle and Zumwalt 1981; Eckblad 1981; Eckblad, Volden, and Weilgart 1984;

ESE 1981; Holland 1986). In addition, natural climatic and hydrologic conditions often overwhelm navigation effects (Johnson 1976).

Ecosystem models are being developed that couple river hydrology and bathymetry to population and community level information on important aquatic resources such as mussels and immature and adult fishes. Quantitative data on freshwater mussels currently exist for a series of sites in the upper Mississippi River (UMR) (Miller and Payne in preparation). Earlier laboratory simulation mussel studies (Aldridge, Payne, and Miller 1987; Payne and Miller 1987) contain a limited number of treatments that do not span the most recent projected traffic levels for the UMR. Additional laboratory simulation studies are needed to improve predictive models applicable to freshwater mussels in the UMR, although it will remain inherently difficult to extend such laboratory data to natural populations in the field.

Native freshwater mussels (family: Unionidae), a resource with cultural, ecological, and economic value, reach their greatest abundance and richness in large and medium-sized rivers in the central United States. These organisms are long-lived (30 or more years for many species) and feed on suspended particulate organic matter. Immediately after being released from the female, immature mussels must spend approximately 10 days on the gills or fins of a freshwater fish. After the mussel drops off, it is virtually nonmotile and spends the rest of its life partially buried in stable sand and gravel or sand and silt substratum. Dense and diverse assemblages of freshwater mussels indicate permanent water, stable substratum, and moderate-to-good water quality (Williams et al. 1992). There are nearly 300 taxa of freshwater mussels in the United States and Canada. Williams et al. (1992) report that 71.7 percent are endangered, threatened, or of special concern.

Laboratory Simulation Studies of Commercial Traffic Effects

Navigation traffic intermittently exposes some freshwater mussels in navigable waterways to turbulence above ambient levels. Factors such as proximity to the navigation channel and seasonal variation in ambient turbulence determine if or to what degree a specific habitat area is affected (Simons et al. 1981; Johnson 1976). In response to concerns about potential adverse effects of gradually increasing commercial navigation traffic rates (Rasmussen 1983), laboratory studies were conducted to specifically evaluate physiological consequences to freshwater mussels of intermittent exposure to turbulence (Aldridge, Payne, and Miller 1987; Payne and Miller 1987) and turbulence plus turbidity (Aldridge, Payne, and Miller 1987). Since those studies, extensive information has been collected on water velocity changes in relation to towboat and barge passage at prominent mussel beds throughout the UMR (Miller et al. 1990, Miller and Payne in preparation).

Laboratory simulation experiments were used previously to investigate the sublethal effects of pulses of suspended sediment and turbulence (Aldridge, Payne, and Miller 1987) and current velocity (Payne and Miller 1987) on species

of freshwater mussels. The indicators of stress were tissue condition index and respiration rate for current velocity studies, plus food clearance rate and nitrogen excretion rates for investigations of suspended sediment effects. Tables 1 and 2 summarize these studies.

Table 1 Summary of Previously Conducted Laboratory Studies on Current Velocity Effects		
Species: <i>Fusconaia ebena</i>		
Size Range: 17-26 mm total shell length		
Treatments		
1	2	3
Continuous low velocity 7 cm/sec	Continuous high velocity 27 cm/sec	5 min high velocity 55 min low velocity
Index of stress: Tissue dry mass:shell dry mass (TDM:SDM) Respiration rate umoles O ₂		
Summary: No effect of treatments on respiration rates Significant (p<0.05) effect on tissue condition in Treatment 3		
Information source: Payne and Miller (1987)		

Each of these studies had treatments that simulated differences in frequency of vessel passage in a waterway. Results demonstrated that moderate levels of traffic did not cause measurable stress in the experimental organisms. However, since these studies were completed, more information on the physical effects of commercial traffic movement has been obtained from modeling studies conducted at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, and better predictions on proposed traffic intensities have been obtained in the UMR. In addition, results of field studies at selected beds (Miller and Payne in preparation) have provided data on physical and biotic conditions at prominent beds in the UMR.

The design of laboratory simulation studies was based upon field measures of velocity changes attributed to vessel passage (Miller, Payne, and Ragland 1990; Miller and Payne in preparation). The objective was to determine if frequency of intermittent exposure to high water velocity is correlated with measurable adverse effects on mussel physiological energetics as assessed by tissue condition index, filtration rate, oxygen uptake, nitrogen excretion, and O:N ratio. In addition, the additive effects of suspended solids were investigated using concentrations and frequencies of disturbance more realistic than those used by Aldridge, Payne, and

Table 2 Summary of Previously Conducted Laboratory Studies on Suspended Solids (SS) and Turbulence Effects			
Species: <i>Quadrula pustulosa pustulosa</i> <i>Fusconaia cerina</i> <i>Pleurobema beadleanum</i>			
Treatments			
1	2	3	4
Infrequent turbulence with SS 750 mg/L SS 7 min/3 hr	Infrequent turbulence without SS 7 min/3 hr	Frequent turbulence with SS 600 mg/L SS 7 min/0.5 hr	Frequent turbulence without SS 7 min/0.5 hr
Indices of stress: TDM:SM Food clearance rates Oxygen uptake rates Nitrogen excretion rates			
Summary: High versus low turbulence only caused significant change in nitrogen excretion rates for three mussel species (Treatments 2 and 4) Infrequent turbulence versus infrequent turbulence plus SS caused significant changes in all indices except TDM:SDM (Treatments 1 and 2) Frequent turbulence versus frequent turbulence plus SS caused significant changes in all indices except TDM:SDM (Treatments 3 and 4)			
Information source: Aldridge, Payne, and Miller (1987)			

Miller (1987). Results of all these laboratory studies have been evaluated, and a set of curves relating stress to magnitude of disturbance have been prepared. These curves can be used in a comprehensive model of commercial navigation effects on freshwater mussels in the UMR.

This work includes information on several other methods for assessing navigation traffic effects. Continuous monitoring of shell gape, a behavioral indicator of disturbance, was conducted in relation to vessel passage in the UMR. In addition, a method of quantifying mussel fecundity was developed that can be applied to condition studies of different unionid species. Studies of fecundity and shell gape behavior can also be used to analyze the physical effects of commercial navigation.

Purpose and Scope

The purpose was to conduct laboratory simulation studies on pulses of turbulence or water velocity and suspended solids. Results of these and previous studies were used to develop curves relating degree of stress to magnitude of disturbance. These curves can be used in a comprehensive model of navigation-traffic effects. A device to monitor mussel shell gape was developed, tested in the laboratory, and used in the field to examine effects of pulses of water velocity on behavior. Finally, a procedure to measure mussel fecundity was developed and used to investigate effects of commercial traffic.

2 Physiological Effects of Intermittent Turbulence

Materials and Methods

Two species were used in these experiments: *Amblema plicata plicata* and *Fusconaia ebena*. Both species are thick shelled and commercially valuable. *Amblema p. plicata* is the most abundant mussel of major mussel beds throughout the UMR and is a species that tolerates impoundment conditions. Several common and abundant fish species serve as host to larval *A. p. plicata*. *Fusconaia ebena* is the dominant unionid of mainstream shoals in the lower Ohio and Tennessee rivers (Williams 1969; Miller, Payne, and Siemsen 1986). The host fish of *F. ebena* larvae is the skipjack herring, *Alosa chrysochloris*. A hydroelectric dam on the UMR at Keokuk, Iowa, prevents upstream migration of *A. chrysochloris*. Prior to construction of this dam, *F. ebena* was the most abundant unionid of major mussel beds throughout the UMR (Coker 1914). Thus, *F. ebena* was the dominant species of mainstream throughout the major inland waterways prior to construction of the modern navigation system.

Amblema p. plicata were collected on 19 July 1995 at river mile (RM) 635.2 of the UMR. Mussels were packed among moist towels above freezer packs in an ice chest and shipped by overnight mail to the laboratory in Vicksburg, MS. Mussels were cleaned of aufwuchs, numbered, and gradually acclimated to dechlorinated tap water at 25 °C. *Fusconaia ebena* were collected at RM 967.6 of the lower Ohio River on 19 October 1995 and treated as above.

Nine mussels from each of two size classes of *F. ebena* (mean shell length (SL) of 35 and 68 mm and tissue dry mass (TDM) of 0.40 and 3.21 g) and one size class of *A. p. plicata* (mean SL and TDM of 74 mm and 2.26 g) were assigned to each of three treatments, involving exposure to a 5-min period of turbulence (high water velocity equal to 45 cm·s⁻¹) once every 0.5, 2.0, or 5.0 hr. Water velocity equaled 11 cm·s⁻¹ in between the brief periods of high water velocity. These treatments were accomplished using three 50-gal oval exposure flumes that were supplied by and drained into a common 300-gal reservoir. Mussels were placed in 7-cm-deep gravel, with siphons downstream. Gravel was covered by 15 cm of water during high flow and 4 cm of water during low flow. Every day approximately 2 percent of the water was removed from the reservoir and replaced with fresh, dechlorinated tap water.

Mussels were removed after 49 days and used in measurements of filtration rate (FR), respiration rate (VO_2), nitrogen excretion rate (NE), and tissue condition index (TCI, equal to the ratio of TDM to shell dry mass). TCI was also determined for each species-size group at the onset of the experiment. The size distribution of mussels for these initial TCI determinations matched the size distribution used in the experiments. Tissues and shell were dried to constant weight at 60 °C.

For FR measurements, each mussel was placed in a 1-L beaker with a known volume (0.25 to 0.5 L, depending on mussel size) of a premixed $5\text{-mg}\cdot\text{L}^{-1}$ suspension of dried *Chlorella*. Temperature was maintained at 25 °C using a water bath, and mussels were allowed to feed for approximately 2 hr. Using a Bausch and Lomb Model 710 spectrophotometer, absorption of 690 nm light was measured for a sample from each mussel's beaker as well as for beakers holding the algal suspension but no mussels. Absorbance was converted to concentration using standard curves made by 0, 30, and 60 percent dilution of the algal suspension. In nearly all instances, less than 40 percent depletion of the algal suspension occurred.

VO_2 and NE were measured by sealing mussels in containers of dechlorinated tap water incubated at 25 °C for approximately 3 hr and comparing to values determined for blanks (water with no mussel). Dissolved oxygen was measured by injecting a water sample into a cell with a polarographic oxygen sensor. Typically, mussels used less than 30 percent of available oxygen. Ammonia concentration was measured using either an ammonia probe (*A. p. plicata*) (Russell-Hunter et al. 1983) or the phenate method (*F. ebena*) (American Public Health Association 1981). Ratios of O:N were computed from the concurrent measurements of VO_2 and NE (Bayne and Newell 1983, Russell-Hunter et al. 1983).

Table 3 summarizes the design of this experiment.

Results

Frequency of high water velocity exposure had little effect on physiological condition in terms of FR, VO_2 , NE, O:N, or TCI (Table 4). All of these aspects of physiological condition contribute to the total bioenergetic budget and might be altered in response to changes in feeding conditions. The only significant inter-treatment difference was observed in FR and occurred only in large *F. ebena*. Large *F. ebena* had a mean FR approximately two times higher when exposed to a 5-min period of high water velocity once per 0.5 hr than once per 2.0 or 5.0 hr.

Despite the lack of inter-treatment differences in final TCI (Table 4), laboratory holding during the 7-week experiment led to a general (i.e., equal among treatments) decline in TCI of small *F. ebena* (Table 5). Differences in final versus initial TCI of small *F. ebena* ranged from 5 to -43 percent, with only 3 of 27 mussels showing increased TCI. Mean percent differences equaled -18, -17, and -22 in low, medium, and high frequency treatments, respectively. These reductions were significant at the 0.07 probability level (ranging from 0.018 in the high frequency to 0.069 in the medium frequency exposure groups). Statistically,

Table 3 Design of Laboratory Studies of Physiological Effects of Intermittent Turbulence		
Species: <i>Amblema plicata plicata</i>		
Size Classes: Small = 35-mm mean shell length Large = 68-mm mean shell length		
Treatments		
1	2	3
Infrequent 5 min per 0.5 hr	Moderately frequent 5 min per 2.0 hr	Frequent 5 min per 5.0 hr
Physiological indicators of stress: Filtration rate (FR) Respiration rate (VO ₂) Nitrogen excretion rate (NE) O:N Tissue condition index (TCI)		
Notes: The 5-min period of turbulence consisted of an increase from an ambient water velocity of 11 cm/sec to a high velocity of 45 cm/sec.		

significant shifts in TCI were not observed in either large *F. ebena* or large *A. p. plicata*, although the tendency was toward reduced TCI. Mean percent difference in final versus initial TCI equaled -7, -9, and -1 in low, medium, and high frequency treatments, respectively. Percent differences equaled -14, -19, and -7 in low, medium, and high frequency treatments, respectively. Individual variation in final TCI of large *A. p. plicata* was high, thus even a 19 percent mean reduction was not statistically significant. Initial TCI of small and large *F. ebena* equaled 2.56 ± 0.19 and 3.56 ± 0.20 . Initial TCI of large *A. p. plicata* equaled 2.79 ± 0.22 .

Concurrent measurement of VO₂ and NE allows insight into patterns of catabolic partitioning. VO₂ of small *F. ebena* averaged $0.40 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ for all treatments and was almost two times higher than the average of $0.22 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ measured for large *F. ebena*. This twofold difference between mussel size classes in weight-specific rates also applied to FR, with the exception of the high FR measured for large *F. ebena* after high frequency exposure. In contrast, small and large *F. ebena* had virtually identical NE. Thus, O:N was approximately two times higher in small (27.9) than large (13.0) *F. ebena*. Rates of VO₂ in large *A. p. plicata* were the same as in large *F. ebena*; however, NE of large *A. p. plicata* ($57 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) was approximately three times higher than in large ($20 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) or small ($17 \mu\text{g} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) *F. ebena*. Thus, ratios of O:N in large *A. p. plicata* were strikingly low, ranging from 5.6 to 6.8. Ultimately, the determination of whether or not shifts in O:N and TCI affect long-term success and survival of mussels in nature must involve field studies.

Table 4
Tissue Condition Index (TCI), Filtration Rate (FR), Respiration Rate (VO₂), Nitrogen Excretion Rate (NE), and O:N Ratios of Mussels Exposed to Brief Periods of High Water Velocity at Low, Medium, and High Frequency

Mussel Group	Variable	Frequency of High Velocity Exposure			ANOVA		
		Low	Medium	High	df	F	p
small <i>Fusconaia ebena</i>	TCI	2.10 ± 0.11	2.13 ± 0.12	2.01 ± 0.09	2, 24	0.3515	0.7072
	FR (mg · g ⁻¹ · h ⁻¹)	0.78 ± 0.18	0.74 ± 0.16	0.81 ± 0.23	2, 24	0.0288	0.9717
	VO ₂ (ml · g ⁻¹ · h ⁻¹)	0.43 ± 0.04	0.39 ± 0.02	0.37 ± 0.04	2, 22	0.6883	0.5129
	NE (μg · g ⁻¹ · h ⁻¹)	17.99 ± 1.14	18.31 ± 1.17	15.25 ± 2.64	2, 22	0.9093	0.4174
	O:N	27.04 ± 1.92	25.40 ± 1.81	31.28 ± 4.00	2, 22	1.2802	0.2979
large <i>Fusconaia ebena</i>	TCI	3.32 ± 0.18	3.24 ± 0.28	3.53 ± 0.14	2, 24	0.5264	0.5974
	FR (mg · g ⁻¹ · h ⁻¹)	0.38 ± 0.10	0.37 ± 0.07	0.77 ± 0.14	2, 24	3.9669	0.0324
	VO ₂ (ml · g ⁻¹ · h ⁻¹)	0.24 ± 0.01	0.22 ± 0.02	0.19 ± 0.01	2, 24	2.1484	0.1386
	NE (μg · g ⁻¹ · h ⁻¹)	19.84 ± 1.01	20.15 ± 1.24	19.18 ± 1.86	2, 24	0.1221	0.8856
	O:N	14.02 ± 1.32	12.65 ± 1.00	12.29 ± 1.41	2, 24	0.5314	0.5945
large <i>Ambalema p. plicata</i>	TCI	2.41 ± 0.49	2.25 ± 0.84	2.58 ± 0.65	2, 24	0.4811	0.6239
	FR (mg · g ⁻¹ · h ⁻¹)	0.33 ± 0.04	0.29 ± 0.07	0.26 ± 0.04	2, 24	0.4140	0.6656
	VO ₂ (ml · g ⁻¹ · h ⁻¹)	0.21 ± 0.02	0.26 ± 0.04	0.20 ± 0.01	2, 24	1.1474	0.3342
	NE (μg · g ⁻¹ · h ⁻¹)	45.57 ± 8.39	77.24 ± 21.59	47.54 ± 9.43	2, 24	1.5104	0.2411
	O:N	6.83 1.22	5.64 1.06	6.49 1.18	2, 24	0.2852	0.7544

Table 5
Comparison of Initial to Final Tissue Condition Index (TCI) Using Student's T-test. Mean Percent Difference in Final Versus Initial TCI is Indicated for Each Treatment; Degree of Freedom Equals 16 for all Comparisons

Mussel Group	Low Frequency Exposure			Medium Frequency Exposure			High Frequency Exposure		
	Mean % Diff	t	p	Mean % Diff	t	p	Mean % Diff	t	p
small <i>Fusconaia ebena</i>	18.0	-2.08	0.054	16.8	-1.95	0.069	21.5	-2.65	0.018
large <i>Fusconaia ebena</i>	6.7	-0.91	0.379	9.0	-0.94	0.361	0.8	-0.14	0.893
large <i>Ambalema p. plicata</i>	13.6	-1.33	0.201	19.2	-1.33	0.170	7.3	-0.64	0.533

Discussion

Not surprisingly, studies of physiological consequences of intermittent disturbance of mussels are rare. Two such studies (Aldridge, Payne, and Miller 1987; Payne and Miller 1987) were conducted in response to concerns about potential adverse consequences to mussels of physical effects of commercial navigation traffic in large inland waterways (Rasmussen 1983). The first such study by Aldridge, Payne, and Miller (1987) measured TDM, FR, VO_2 , NE, and O:N of three species of mussels (*Quadrula pustulosa*, *Fusconaia cerina*, and *Pleurobema beadleanum*) exposed for 9 days to a 7-min disturbance once per 0.5 hr and once per 3.0 hr; disturbance involved either turbulence alone or turbulence plus very high suspended inorganic solids concentrations. The turbulence portion of their experiments is especially valuable for comparison to the first part of the present study. All three species had significantly lower NE when exposed frequently versus infrequently; one species (*P. beadleanum*) showed a significant increase in O:N. A second study was conducted by Payne and Miller (1987) in which juvenile *F. ebena* were exposed for approximately 5 weeks to either continuous low (7 cm/sec), continuous high (27 cm/sec), or intermittent high (a 5-minute period once per hour) water velocity. Assessments were made of TCI and VO_2 . Reduction of TCI averaged 20 and 22 percent for mussels in continuous low- and intermittent high-velocity treatments, respectively, reflecting a general effect of laboratory holding. This level of reduction is similar to that observed in small *F. ebena* in the present study. However, significantly greater reduction (35 percent) was measured in mussels continuously exposed to high water velocity. Thus, despite the general decline of 20-22 percent in the other two treatments, the additional physical stress of the continuous high-velocity treatment elicited an additive reduction in TCI. Continuous but not intermittent exposure to high water velocity had a negative effect relative to continuous low water velocity exposure.

Essentially the same suite of physiological factors as used by Aldridge, Payne, and Miller (1987) were used in the present study, and the experiment ran much longer than that of Aldridge, Payne, and Miller (1987) and slightly longer than that of Payne and Miller (1987). Nonetheless, a graded response to frequency of intermittent exposure to high water velocity was not detected in terms of FR, VO_2 , NE, O:N, nor TCI. The only significant effect of frequency of exposure was on FR of large *F. ebena*; this result was anomalous in that substantially higher FR was measured for those mussels exposed most frequently to brief periods of high water velocity.

Recent field studies by Miller et al. (1990) and Miller and Payne (in preparation) were the first to quantify physical effects of commercial navigation traffic specifically in relation to prominent mussel beds. Results of these field studies were considered in the design of the present laboratory study. In the present laboratory study, ambient velocity (11 cm/sec) was increased by a factor of approximately 4 (to 45 cm/sec) during intermittent periods of high water velocity. Miller and Payne (in preparation) measured effects of 60 passages of commercial vessels with the following results: 20 percent had major effects (typically a two- to three-fold increase or decrease in ambient velocity), 37 percent had minor effects, and 43 percent had no effect. Similarly, ESE (1981) reported twofold changes in water velocity as a major effect and observed no measurable change in relation to

8 of 23 barge passages in the upper Mississippi River. The greatest changes reported by Miller and Payne (in preparation) for upbound towboat and barge passage was a shift from 11 to 22 cm/sec; the greatest effect of a downbound passage was a shift from 18 to 55 cm/sec.

Each episode of high-velocity exposure in the present laboratory study lasted 5 min -- a duration longer than that of 3 min typically experienced by natural populations of mussels (Miller and Payne in preparation). Also, two of the frequencies of disturbance used in the present laboratory study exceeded those likely to be encountered by naturally occurring mussels. The average number of barge tows per day varies with location and year, ranging from approximately 6-12 in the upper Mississippi River (Miller and Payne in preparation). However, only approximately 60 percent of barge passages cause measurable velocity changes in the field (ESE 1981, Miller and Payne in preparation). Thus, maximum frequency of exposure in the field is likely to average one measurable event per 3.3 hr. This frequency is similar to the infrequent exposure treatment used by Aldridge, Payne and Miller (1987) but more than three times lower than that used as an intermittent exposure treatment by Payne and Miller (1987). The frequencies of disturbance used in the present laboratory study (once per 0.5, 2.0, or 5.0 hr) span the range both of previous laboratory studies and situations likely to be encountered in the field.

Neither results presented herein nor those of Aldridge, Payne, and Miller (1987) or Payne and Miller (1987) indicate sustained negative physiological effects of intermittent exposure to high water velocity at frequencies likely to be encountered in association with routine commercial navigation traffic. Recent field studies provided not only perspective on the frequency of such disturbances, but also allowed more realistic design than earlier studies with respect to the magnitude and duration of exposure.

Frequency of intermittent exposure to turbulence had no effect on mussel physiological condition. Although mussels at all exposure frequencies showed significantly reduced TCI compared to initial condition, there were no differences between low-, medium-, and high-frequency treatments. Thus, the TCI decline reflects a general bioenergetic stress of laboratory holding in this moderately long-term experiment in which no additive effects of turbulence were observed.

Ratios of O:N reflect differential catabolism of energy reserves. Values are low if protein is catabolized and high if lipid or starch is catabolized. Theoretical calculations of O:N protein catabolism vary slightly depending on the elemental composition assumed for protein, ranging from 7 to 9 (Conover and Corner 1968, Snow and Williams 1971, Bayne 1973, Mayzaud (1973). Metabolic coefficients of 0.94, 2.04, and 0.84 are commonly used to estimate O₂ consumed (in liters) per gram of protein, lipid, or carbohydrate catabolized (West and Todd 1961). One liter O₂ yields 0.09205 moles O at 25 °C and a pressure of 1 atmosphere. In addition, 0.0114 moles N (0.16 g) is excreted per gram protein catabolized (West and Todd 1961). Using these coefficients, it can be calculated that metabolic oxidation of protein and carbohydrate at weight ratios of 1:1, 1:3, and 1:10 leads to an expected O:N of 14, 28, and 75, respectively. Catabolism of protein and lipid at

weight ratios of 1:1, 1:3, and 1:10 would yield an O:N of 24, 57, and 173, respectively.

Well-fed animals in nature or the laboratory vary their assimilation and anabolic versus catabolic uses of different biochemical components of the food, making O:N ratios more difficult to interpret than in starved animals. For example, O:N of marine copepods varied from 12 to 50 despite a stable C:N of 6 for suspended particulate matter consumed by these animals. In the present study, mussels were fed dried *Chlorella* to stimulate filtration and help satisfy metabolic costs. The approximate protein:lipid:carbohydrate ratio of dried *Chlorella* is 3.5:1.0:1.0.¹ An O:N value of approximately 14 would be expected based on complete metabolic oxidation of this food.

The simple diet of dried *Chlorella* certainly was not nutritionally sufficient. Negative bioenergetic balance was indicated by significant reduction in TCI in small *F. ebena* by the end of the 7-week experiment and the tendency toward such reduction in large *F. ebena* and *A. p. plicata*. Thus, O:N ratios of all mussel groups probably reflected catabolism of endogenous substrates in addition to food.

The higher O:N values of small versus large *F. ebena* suggested greater reliance on nonprotein energy reserves in the small mussels. An alternative and not mutually exclusive scenario is that deamination of protein components of food was reduced in small versus large mussels, perhaps indicating a higher premium on amino acid conservation to support future tissue growth of the small mussels. Assuming total oxidation of all biochemical components of dried *Chlorella* and supplemental catabolism of stored glycogen or galactogen (typically the energy reserve of freshwater molluscs), the O:N ratio of 28 observed in small *F. ebena* corresponds to a protein: lipid: carbohydrate ratio of 3.5: 1.0: 8.1. The latter ratio in turn corresponds to a catabolic substrate ratio of 1.0 g *Chlorella* to 1.3 g stored carbohydrate and is concordant with the 17 to 22 percent TCI reduction of small *F. ebena*. In contrast, values of O:N for large *F. ebena* (13-14) closely corresponded to the value of 14 expected if O:N reflected only the complete catabolism of dried *Chlorella*. In addition, the moderate and statistically insignificant TCI reduction of large *F. ebena* indicated that food-based catabolism more nearly satisfied the metabolic needs of large mussels. *Fusconaia ebena* were collected in fall when prewinter build-up of nonprotein energy reserves is common.

Very low values of O:N for large *A. p. plicata* (6-8) reflected catabolism of essentially pure protein, possibly indicated supplementation of food-based catabolism with degradation of tissue protein. An alternate and not mutually exclusive scenario is that assimilated food was incompletely catabolized, with carbon chains of deaminated amino acids, carbohydrates, or lipids having been partially used to build nonprotein energy stores (Aldridge, Russell-Hunter, and McMahon 1995). Large *A. p. plicata* were collected from the upper Mississippi River in mid-July, probably in recent post-spawning condition and thus possibly depleted of non-protein energy reserves.

¹ Personal Communication, 1997, Dan Hornbach, Macalester College, St. Paul, MN.

In summary, measurements of TCI, FR, VO_2 , NE, and O:N were meaningful indicators of mussel physiological condition. Joint evaluation of TCI and O:N indicated bioenergetic stress associated with this moderately long-term laboratory experiment. TCI and O:N indicated size and species differences in both availability of endogenous energy reserves and in the ability of food assimilation to meet concurrent metabolic needs. Comparison of physiological condition among frequency of exposure treatments did not indicate physiological effects of intermittent turbulence in that none added to the bioenergetic stress of moderately long-term laboratory holding.

3 Effects of Suspended Solids and Turbulence

Materials and Methods

Experimental manipulation of total suspended solids (TSS)

A series of preliminary studies was conducted using sediment from Brown's Lake (Vicksburg, MS) to determine how to create desired TSS concentrations in addition to intermittent turbulence (Experiments I and II of this part). Sediment was collected, oven dried at 105 °C, and sorted using a USGS sieve series. After several trials, it was determined that sediments passing the 200 and 230 sieves could be used to maintain a 20-mg/L suspension during periods of either high or low water velocity. These fine sediment fractions were also used to create a continuous concentration of 80-120 mg/L with continuous low, medium, or high water velocity (Experiment III of this part). Slightly coarser grained sediments were used in high suspended solids treatment to allow TSS to rise to 120 mg/L during high-water-velocity periods and then rapidly decline to 20 mg/L during periods of low velocity.

Programmable timers and submersed water pumps were used to create desired water velocity conditions. Both during laboratory holding and the 2-week experiments, water temperature was maintained at 20 °C and mussels were fed a 2 mg/L suspension of dried *Chlorella* twice per week. Tables 6-8 summarize the design of Experiments I, II, and III.

Experiment I: Frequent turbulence with high versus low TSS

Amblema p. plicata, *Plectomerus dombeyanus*, and *Quadrula pustulosa* used were collected from the Sunflower River, Mississippi, on 22 June 1996. Mussels were packed among moist towels above in an ice chest and returned to the laboratory in Vicksburg, MS. Mussels were cleaned of aufwuchs, numbered, and gradually acclimated to dechlorinated tap water at 20 °C.

A total of 28 *A. plicata*, 28 *P. dombeyanus*, and 11 *Q. pustulosa* were used in the Phase 1 experiments, split as equally, by number and size, as possible between two treatments. These treatments were high-frequency turbulence (5 min every 30 min) with low versus high suspended solids. In the high-suspended-solids treatment, total suspended solids averaged 120 mg/L during the 5-min periods of

Table 6**Design of Experiment I: Effects of Frequent Exposure to Total Suspended Solids and Turbulence**

Species	Treatment n	Control n	Initial Condition n
<i>Amblema p. plicata</i>	14	14	8
<i>Plectomerus dombeyanus</i>	14	14	8
<i>Quadrula p. pustulosa</i>	6	7	6
Treatment - ~ 120 mg/L suspended sediments Control - ~ 20 mg/L suspended sediments Frequency - 5 min of every 30 min Duration - 14 days			
Variables measured: Filtration rate Oxygen consumption Nitrogen excretion Tissue mass TCI O:N ratio			

high water velocity (50 cm/sec) and fell, within 5 min, to approximately 20 mg/L during the intervening 25-min periods of low water velocity (7 cm/sec). TSS in the low-suspended-solids treatment ("control") was maintained constantly at approximately 20 mg/L.

Mussels were glued to the walls of the exposure chambers (50-gal oval flumes), exposed to treatment conditions for 14 days, and then used in a series of physiological rate measurements. Respiration, ammonia excretion, and filtration rates were measured for each individual. Mussels were then measured for shell length (SL), tissues were separated from shells, and the dry weight of both components were measured after oven-drying at 60 °C to constant weight. Tissue condition indices were calculated as ratios of tissue mass to shell mass and tissue mass to shell length.

Experiment II: Infrequent turbulence with high versus low TSS

Amblema p. plicata, *Plectomerus dombeyanus*, and *Quadrula pustulosa* used were collected from the Sunflower River, Mississippi, on 11 July 1996. Mussels were handled as in the first experiment. A total of 28 *A. plicata*, 28 *P.*

Table 7
Design of Experiment II: Effect of Infrequent Exposure to Suspended Solids and Turbulence

Species	Treatment n	Control n	Initial Condition n
<i>Amblyema plicata</i>	14	14	14
<i>Plectomerus dombeyanus</i>	14	14	14
<i>Quadrula pustulosa</i>	9	9	9
Treatment - ~ 120 mg/L suspended sediments Control - ~ 20 mg/L suspended sediments Frequency - 5 min of every 180 min Duration - 14 days			
Variables measured: Filtration rate Oxygen consumption Nitrogen excretion Tissue mass TCI O:N ratio			

dombeyanus, and 18 *Q. pustulosa* were used in the Phase 2 experiment, split as equally, by number and size, as possible between two treatments. These treatments were low-frequency turbulence (5 min every 3 hr) with low versus high suspended solids. In the high-suspended-solids treatment, as previously described, mussels were positioned in the exposure flumes and assessed for condition and physiological rates as described for the first experiment.

Experiment III: Continuous low, medium, and high water velocity with high TSS

Using different combinations of pump sizes and pipe diameters, mussels were exposed continuously to mean water velocities of either 7, 25, or 50 cm/sec and TSS of 80-120 mg/L for 14 days. Mussels were handled as described above, with the same series of condition and physiological rate assessments made at the end of the experiment. A total of 30 *A. p. plicata*, 24 *P. dombeyanus*, and 24 *Q. pustulosa* were split equally among the three treatments. Mussels used in this experiment were collected from the Sunflower River on 22 August 1996.

Table 8 Design of Experiment III: Effect of High Total Suspended Solids Exposure at Three Different Levels of Turbulence			
Species	Treatment n	Control n	Initial Condition n
<i>Amblema p. plicata</i>	14	14	14
<i>Plectomerus dombeyanus</i>	14	14	14
<i>Quadrula p. pustulosa</i>	10	10	10
Treatments - ~ 120 mg/L suspended sediments, 7 cm/sec ~ 120 mg/L suspended sediments, 30 cm/sec ~ 120 mg/L suspended sediments, 60 cm/sec Frequency – continuous flow Duration – 14 days			
Variables measured: Filtration rate Oxygen consumption Nitrogen excretion Tissue mass TCI O:N ratio			

Physiological rate measurements

FR, VO_2 , and NE measurements were made for each mussel according to methods described in Chapter 2. Mussels were incubated in water at 20 °C for these rate measurements.

Results

Experiment I: Frequent turbulence with high versus low TSS

High versus low suspended solids exposure during frequent turbulence exposure led to significant physiological shifts in both *P. dombeyanus* and *Q. pustulosa* but not *A. p. plicata* (Table 9). In *P. dombeyanus*, ammonia excretion was 25 percent less after 14 days exposure to frequent turbulence with high TSS than after the same length exposure to frequent turbulence with low TSS. Ratio of O:N was 19 percent higher under high versus low suspended solids exposure. In *Q. pustulosa*, significant differences were noted in respiration, ammonia excretion, and O:N. Respiration rates were 42 percent less after high versus low TSS

Table 9
Results of Experiment I: Effects of Frequent Exposure to Suspended Solids and Turbulence

Species	Variable	Suspended Sediment Treatment		T-tests		
		Control	High	df	t	P
<i>A. p. plicata</i>	TCl a	5.49 ± 0.46	5.24 ± 0.39	26	-0.453	0.6539
	TCl b	6.97 ± 0.15	6.86 ± 0.35	26	-0.188	0.8519
	FR (mg/g/hr)	0.07 ± 0.01	0.06 ± 0.01	26	-0.559	0.5812
	VO ₂ (ml/g/hr)	0.14 ± 0.01	0.15 ± 0.01	26	0.459	0.6497
	NE (µg/g/hr)	16.86 ± 1.36	18.23 ± 1.65	26	0.642	0.5261
	O:N	11.15 ± 0.99	10.26 ± 0.41	26	-0.828	0.4153
<i>P. dombeyanus</i>	TCl a	5.64 ± 0.27	6.02 ± 0.37	26	0.828	0.4153
	TCl b	6.49 ± 0.41	6.51 ± 0.03	26	0.032	0.9748
	FR (mg/g/hr)	0.05 ± 0.00	0.04 ± 0.00	26	1.035	0.1892
	VO ₂ (ml/g/hr)	0.16 ± 0.01	0.14 ± 0.01	26	-1.095	0.2834
	NE (µg/g/hr)	32.47 ± 2.04	24.47 ± 2.03	26	-2.777	0.0100
	O:N	5.99 ± 0.27	7.11 ± 0.24	26	3.118	0.0044
<i>Q. p. pustulosa</i>	TCl a	2.78 ± 0.17	3.62 ± 0.25	9	2.824	0.0199
	TCl b	2.35 ± 0.23	2.64 ± 0.23	9	0.855	0.4145
	FR (mg/g/hr)	0.08 ± 0.02	0.09 ± 0.02	9	0.174	0.8658
	VO ₂ (ml/g/hr)	0.24 ± 0.03	0.14 ± 0.02	9	-2.803	0.0206
	NE (µg/g/hr)	18.34 ± 3.80	1.96 ± 1.12	9	-3.780	0.0043
	O:N	18.85 ± 2.24	89.97 ± 33.88	7	2.278	0.0568

exposure. Nitrogen excretion rates were 89 percent less after high versus low TSS exposure. Consequently, O:N was almost five times greater after high versus low TSS exposure.

FR was moderately high (0.084 to 0.088 mg/g/hr) in *A. p. plicata* and was highly invariant (Table 9). The ratio of SE-to-mean FR was only 0.08 in this species. FR was lower in *P. dombeyanus* (0.035 to 0.044 mg/g/hr) but was similarly invariant (SE-to-mean <0.12). FR was highest in *Q. pustulosa*, and highly variable. Ratios of SE-to-mean FR in *Q. pustulosa* exceeded 0.28.

Respiration rates ranged from slightly less than 0.15 ml O₂/g/hr in both *A. p. plicata* and *P. dombeyanus* to 0.233 ml O₂/g/hr in the smaller *Q. pustulosa* (Table 9). Respiration rate measurements were relatively invariant regardless of species or experimental treatment. In no instance did the ratio of SE-to-mean exceed 0.10.

Nitrogen excretion rates were lowest in *A. p. plicata* (17 to 18 ug/g/hr and highest in *P. dombeyanus* (24 to 32 ug/g/hr)(Table 9). In no instance did SE-to-mean ratio exceed 0.12 for nitrogen excretion rates. Thus, like respiration rate data, these results were relatively invariant within each species-treatment combination.

Ratios of O:N were lowest in *P. dombeyanus* (6.0 to 7.1) and highest in *Q. pustulosa* (18.9 to 90.0) (Table 9). Except for the high-suspended-solids treatment with respect to *Q. pustulosa*, all of these ratios reflect a substantially protein-based catabolism. The ratio of 90.0 observed in *Q. pustulosa* after high-suspended-solids exposure reflected a shift toward a less protein-based (i.e., carbohydrate or lipid) metabolism. Ratios of SE-to-mean O:N ranged from 0.12 in *Q. pustulosa* after low TSS exposure to 0.38 in the same species after high TSS exposure.

In none of the species was there a reduction in TCI due to high versus low TSS exposure (Table 9). Indeed, the only significant difference was noted in *Q. pustulosa*, in which TCI was significantly higher after high versus low TSS exposure. Given the small sample sizes of *Q. pustulosa*, significant differences noted for this species should be viewed cautiously.

Experiment II: Infrequent turbulence with high versus low TSS

Significant physiological shifts were elicited in both *A. p. plicata* and *P. dombeyanus*, but not *Q. pustulosa* (Table 10). In *A. p. plicata*, nitrogen excretion rates were 38 percent lower after high TSS exposure. In addition, O:N ratio was nearly twice as high after high TSS exposure. In *P. dombeyanus*, the only significant shift was toward slightly lower nitrogen excretion after high versus low TSS exposure. None of the species showed a significant reduction in TCI due to high versus low TSS exposure combined with infrequent turbulence.

Filtration rates were highest in *Q. pustulosa* (0.090 to 0.184 mg/g/hr), were intermediate in *A. p. plicata* (0.84 to 0.88 mg/g/hr), and were lowest in *P. dombeyanus* (0.035 to 0.044) (Table 10). As in the previous experiment, filtration rate was highly variable in *Q. pustulosa* (SE-to-mean ranged from 0.29 to 0.57). In contrast, filtration rates were relatively invariant in the other two species (SE-to-mean less than 0.11).

Respiration rates were highest in the smallest species, *Q. pustulosa* (0.233 ml/g/hr), and ranged from 0.125 to 0.149 in the other two species (Table 10). Respiration rates were invariant among individuals of all species. Ratios of SE-to-mean did not exceed 0.10. Nitrogen excretion rates were similarly invariant among individuals. The highest rates were recorded for *Q. pustulosa* (29 to 33 ug/g/h) (Table 10). Rates in *P. dombeyanus* were

Table 10
Results of Experiment II: Infrequent Exposure to Suspended Solids and Turbulence

Species	Variable	Suspended Sediment Treatment		T-tests		
		Control	High	df	t	P
<i>A. p. plicata</i>	TCI a	5.30 ± 0.40	6.20 ± 0.30	26	1.605	0.1206
	TCI b	6.86 ± 0.63	7.60 ± 0.42	26	1.180	0.2487
	FR (mg/g/hr)	0.088 ± 0.007	0.084 ± 0.007	26	-0.340	0.7368
	VO ₂ (ml/g/hr)	0.149 ± 0.013	0.141 ± 0.010	26	0.426	0.6738
	NE (µg/g/hr)	16.716 ± 1.475	10.312 ± 1.234	26	-3.329	0.0026
	O:N	11.542 ± 1.048	19.620 ± 2.831	26	2.670	0.0127
<i>P. dombeyanus</i>	TCI a	5.60 ± 0.20	5.70 ± 0.30	26	0.261	0.7964
	TCI b	6.42 ± 0.20	6.31 ± 0.34	26	-0.379	0.7074
	FR (mg/g/hr)	0.035 ± 0.004	0.044 ± 0.003	26	1.903	0.0680
	VO ₂ (ml/g/hr)	0.139 ± 0.007	0.125 ± 0.006	26	-1.542	0.1353
	NE (µg/g/hr)	22.714 ± 1.463	18.140 ± 0.978	26	-2.599	0.0152
	O:N	8.009 ± 0.576	8.850 ± 0.462	26	1.140	0.2647
<i>Q. p. pustulosa</i>	TCI a	3.00 ± 0.40	2.90 ± 0.20	16	-0.346	0.7341
	TCI b	2.74 ± 0.17	2.64 ± 0.17	16	-0.973	0.3449
	FR (mg/g/hr)	0.090 ± 0.051	0.184 ± 0.053	16	1.260	0.2259
	VO ₂ (ml/g/hr)	0.233 ± 0.021	0.233 ± 0.012	16	0.005	0.9958
	NE (µg/g/hr)	28.843 ± 2.509	32.928 ± 2.200	16	1.225	0.1192
	O:N	10.329 ± 0.714	9.010 ± 0.440	16	-1.572	0.1354

intermediate, ranging from 18 to 23 ug/g/hr. *Amblema p. plicata* showed the lowest nitrogen excretion rates, ranging from 10 to 17 ug/g/hr.

Ratios of O:N ranged from approximately 30 in *Q. pustulosa*, regardless of treatment, to 10 to 16 in *A. plicata*. None of the ratios were sufficiently high to indicate a fundamental shift from protein-based to lipid or carbohydrate based catabolism. Furthermore, ratios were invariant among individuals. The highest SE-to-mean ratio (0.14) was observed in *A. plicata* after exposure to high suspended solids.

No significant differences among treatments were noted for any of the three species.

Experiment III: Continuous high TSS exposure at three distinct water velocities

Cast against a background of continuous high TSS exposure, differences in water velocity conditions led to few physiological differences in any of the three species (Table 11). The only significant inter-treatment differences were noted in *A. plicata* (low filtration rate after high velocity exposure) and *Q. pustulosa* (high nitrogen excretion and low O:N after intermediate-water-velocity exposure). Once again, it was noteworthy that none of the experimental treatments elicited a significant difference in TCI.

Filtration rates were highest in *Q. pustulosa* (0.22 to 0.26 mg/g/hr), intermediate in *A. plicata* (0.08 to 0.13 mg/g/hr), and lowest in *P. dombeyanus* (0.05 to 0.08 mg/g/hr)(Table 11). As noted already, rates were significantly lower in *A. plicata* after high-velocity exposure. However, this difference was not accompanied by any other apparent physiological shifts. SE-to-mean ratios tended to be higher in *Q. pustulosa* than in the other two species.

Respiration rates were highest in *Q. pustulosa* (0.43 to 0.47 ml/g/hr), intermediate in *P. dombeyanus* (0.22 to 0.25 ml/g/hr), and lowest in *A. plicata* (0.12 to 0.15 ml/g/hr) (Table 11). Respiration rates were somewhat more variable among individual *A. plicata* than in the previous two experiments. SE-to-mean ratios ranged from a high of 0.19 in *A. plicata* (low and intermediate water velocity) to 0.05 in *P. dombeyanus* (high-water-velocity exposure).

Nitrogen excretion rates were highest in *Q. pustulosa* (30 to 50 ug/g/hr), intermediate in *A. p. plicata* (21 to 23 ug/g/hr), and lowest in *P. dombeyanus* (12 to 15 ug/g/hr)(Table 11). The low rates observed in *P. dombeyanus* were associated with slightly higher than usual variance among individuals. Ratios of SE-to-mean nitrogen excretion ranged from 0.16 (high velocity) to 0.21 (intermediate velocity) in this species in this experiment.

Faced with continuous exposure to high TSS, the level of continuous exposure to turbulence was unimportant to mussels. None of the species, regardless of treatment, showed a fundamental shift in O:N ratio (Table 11). Regardless of turbulence level, all mussels exposed continuously to high TSS maintained relatively protein-based catabolism.

Discussion

Experiments I and II provided somewhat equivocal evidence that TSS levels affect mussel physiological response to intermittent turbulence. Physiological disruption was slightly greater when high TSS concentration accompanied intermittent turbulence than when low TSS concentration accompanied intermittent turbulence. Faced with infrequent disruption, both *P. dombeyanus* and *A. p. plicata*, but not *Q. pustulosa*, excreted nitrogen at a slightly but significantly lower

Table 11
Results of Experiment III: Effects of Continuous Exposure to High Suspended Solids at Three Water Velocities

Species	Variable	Velocity Treatment			ANOVA		
		Low	Medium	High	df	F	p
<i>A. p. plicata</i>	TCl a	5.180 ± 0.575	5.319 ± 0.518	5.437 ± 0.527	2, 27	0.063	0.9393
	TCl b	6.125 ± 0.696	6.341 ± 0.562	6.688 ± 0.635	2, 27	0.224	0.8009
	FR (mg/g/hr)	0.122 ± 0.019	0.134 ± 0.019	0.078 ± 0.011	2, 27	3.421	0.0474
	VO ₂ (ml/g/hr)	0.150 ± 0.029	0.135 ± 0.025	0.122 ± 0.014	2, 27	0.393	0.6788
	NE (µg/g/hr)	23.337 ± 2.083	20.774 ± 1.448	21.512 ± 2.636	2, 27	0.434	0.6525
	O:N	7.966 ± 1.333	8.387 ± 1.610	7.755 ± 0.908	2, 27	0.066	0.9358
<i>P. dombeyanus</i>	TCl a	5.206 ± 0.272	5.881 ± 0.528	4.908 ± 0.734	2, 24	0.925	0.4101
	TCl b	6.142 ± 0.345	7.584 ± 0.712	6.587 ± 0.487	2, 24	2.289	0.1230
	FR (mg/g/hr)	0.078 ± 0.012	0.071 ± 0.008	0.054 ± 0.011	2, 24	1.364	0.2747
	VO ₂ (ml/g/hr)	0.248 ± 0.023	0.0223 ± 0.019	0.219 ± 0.010	2, 24	0.824	0.4506
	NE (µg/g/hr)	14.888 ± 3.141	15.840 ± 3.322	11.667 ± 1.859	2, 24	0.644	0.5343
	O:N	26.033 ± 3.792	22.042 ± 4.016	27.951 ± 4.370	2, 24	0.576	0.5696
<i>Q. p. pustulosa</i>	TCl a	3.315 ± 0.380	2.970 ± 0.304	2.872 ± 0.324	2, 16	0.516	0.6064
	TCl b	2.748 ± 0.252	2.713 ± 0.242	2.479 ± 0.224	2, 16	0.350	0.7096
	FR (mg/g/hr)	0.261 ± 0.074	0.224 ± 0.042	0.226 ± 0.020	2, 16	0.173	0.8426
	VO ₂ (ml/g/hr)	0.454 ± 0.050	0.429 ± 0.026	0.472 ± 0.055	2, 16	0.228	0.7612
	NE (µg/g/hr)	30.033 ± 3.484	49.809 ± 4.204	30.611 ± 5.687	2, 16	8.492	0.0031
	O:N	19.505 ± 1.627	10.940 ± 0.498	20.701 ± 2.905	2, 16	12.285	0.0006

rate when turbulence was accompanied by high rather than low TSS. For *A. p. plicata*, this was accompanied by a less than twofold increase in O:N under high versus low TSS. Faced with frequent disruption, both *P. dombeyanus* and *Q. pustulosa*, but not *A. p. plicata*, showed similar shifts. The O:N shift in *Q. pustulosa* reflected a fourfold higher ratio with high rather than low TSS.

Thus, the tendency was for downward shifts in NE and upward shifts in O:N. However, this tendency was not manifest in all species within an experiment nor in both experiments for a given species. It is especially perplexing that *A. p. plicata* results indicated a significant additive effect of TSS to turbulence with

infrequent but not frequent disturbance. If shifts seen during infrequent exposure were indeed indicative of physiological changes due to high TSS in addition to turbulence, it is illogical that such effects would have been greater with less rather than more frequent perturbation (Aldridge, Payne, and Miller 1987). That shifts observed were minor and, in the case of *A. p. plicata*, showed a counter-intuitive pattern between experiments makes difficult a straightforward biological interpretation. Although a few statistically significant shifts were noted, it is noteworthy that major changes in metabolic condition generally were not indicated in either Experiment I or II. The O:N shift seen in *Q. pustulosa* in Experiment II was the only change seemingly indicative of a substantial metabolic shift. No changes in tissue condition occurred.

Earlier experiments conducted by Aldridge, Payne, and Miller (1987) were similar to Experiments I and II of the present study with some noteworthy specific differences. High concentrations of TSS used in the present study (120 mg/L) were more realistic than concentrations (600-750 mg/L) used by Aldridge, Payne, and Miller. Likewise, lake sediment used for TSS in the present study was more realistic than diatomaceous earth used by Aldridge, Payne, and Miller. Instead of low TSS for comparison to high TSS, the study by Aldridge, Payne and Miller used no TSS as a control treatment. The duration of the present experiments (14 days) was slightly longer than the 10-day duration used by Aldridge, Payne, and Miller.

The study by Aldridge, Payne, and Miller yielded more substantial and consistent results on effects of TSS added to intermittent turbulence. After frequent exposure to turbulence and high TSS, O:N ratio was 2-14 times higher, depending on species, than after frequent exposure to turbulence without TSS. This magnitude of shift was only noted for *Q. pustulosa* in the present study after exposure to frequent turbulence with high TSS relative to frequent exposure to turbulence with low TSS. Aldridge, Payne, and Miller also observed their greatest level of shift in *Q. pustulosa*.

The generally greater clarity of results obtained by Aldridge, Payne, and Miller probably reflects the more severe level of stress to which they subjected mussels. The lower concentrations of TSS and more natural particles used in the present study probably were less stressful, and, consequently, physiological responses were not as clear. It is probably the case that the more realistic a laboratory experiment becomes with respect to mimicking the ephemeral and somewhat subtle nature of physical effects associated with routine commercial navigation traffic, the less obvious and straightforwardly interpretable are the physiological responses of mussels.

Quantitative relationships of physiological stress to turbulence or turbulence plus suspended solids are suggested from the combined results of studies reported here as well as the previously published results of Aldridge, Payne and Miller (1987) and Payne and Miller (1987). Comparing across studies and experiments, it was necessary to devise a simple index of stress that allows a variety of results to be combined and applied in a singular, purposeful fashion. Experimental treatments that led to no measurable physiological rate changes are considered to reflect conditions causing no stress. A slight stress was indicated if experimental

treatments caused at least some physiological rate shifts in at least some species. Significant but not marked changes in O:N ratio, presumably a precursor of slow shifts in tissue condition index, were indicative of moderate stress. The highest stress levels were indicated by either significant and marked changes in O:N ratios (in short-term experiments) or significant reductions in tissue condition index (in long-term experiments).

Stress Curves for Future Model Development

Syntheses of all experimental results led to preparation of stress curves shown in Figures 1-4. The first curve (Figure 1) is for a low level of turbulence not accompanied by significant suspended solids increase. A low level of turbulence corresponds to a significant but less than 1.6-fold increase above ambient conditions. Experimental results strongly suggest that only with extremely frequent events (2 per hour or 48 per day) does measurable stress begin to become manifest. With increasing frequency of intermittent disturbance, stress presumably rises to some higher, but still only moderately stressful, level. Once a low level of turbulence increase is essentially continuous, it is highly likely that mussels adapt and stress actually decreases below the moderate level associated with very frequent disruptions that are still sensed as discontinuous. The second curve (Figure 2), for high turbulence (approximately doubling above ambient levels), indicates a fundamentally different response. Experimental results still indicate that stress is only low-to-moderate even with two events per hour. However, mussels are unlikely to adapt to continuous high levels of turbulence (e.g., condition index declined under such conditions (Payne and Miller 1987)), and stress achieves maximum level.

The third curve (Figure 3), for high turbulence with high suspended solids, is similar to the second but indicates the clear, additive effect of increased suspended solids concentrations. Thus, at a frequency of two events per hour, stress is moderately high - consistent with results presented herein. Finally, the most stressful conditions were reflected in experiments combining high turbulence and very high suspended solids concentrations (Figure 4). Results of studies by Aldridge, Payne, and Miller (1987) clearly show low-to-moderate stress at a frequency of one event per three hours and maximum stress with two events per hour. These four curves represent a synthesis of results of all laboratory experiments conducted at WES on the effects of passage of commercial vessels on freshwater mussels.

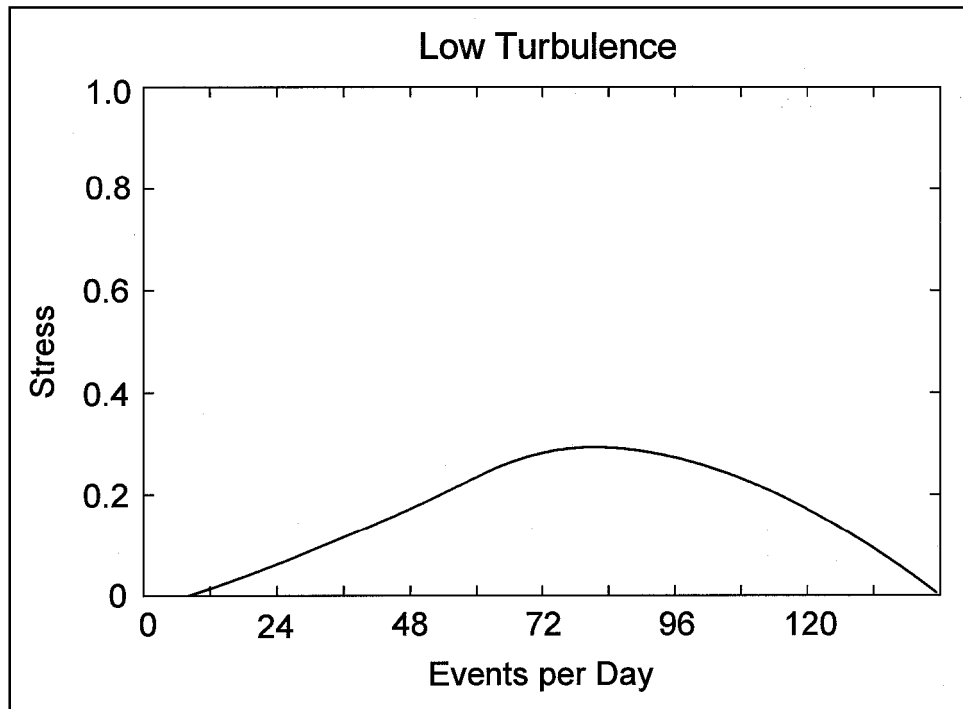


Figure 1. Effects of low levels of turbulence on freshwater mussels

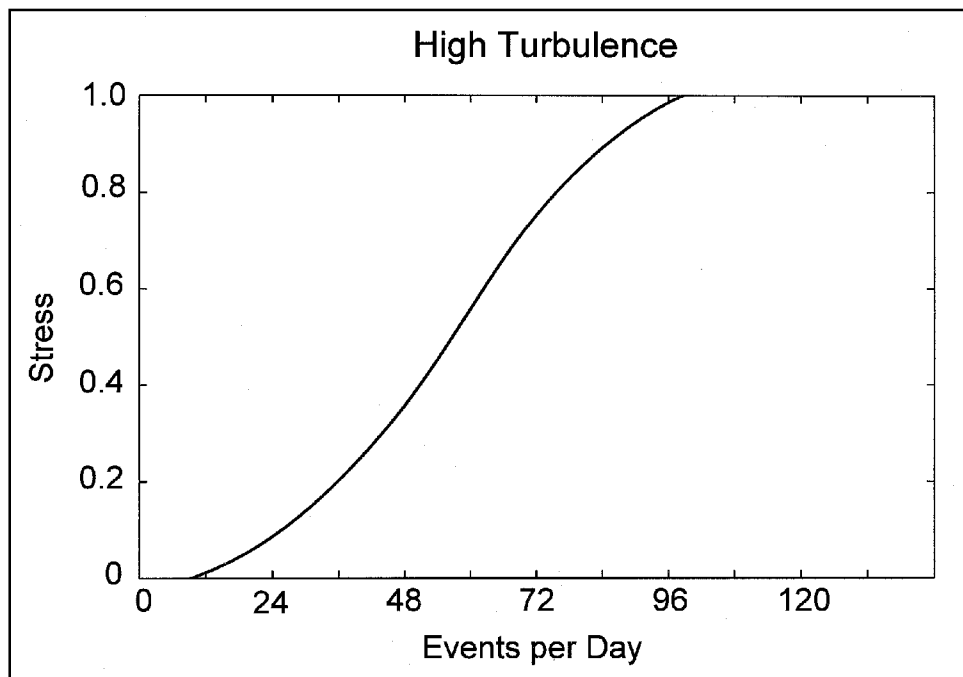


Figure 2. Effects of high levels of turbulence on freshwater mussels

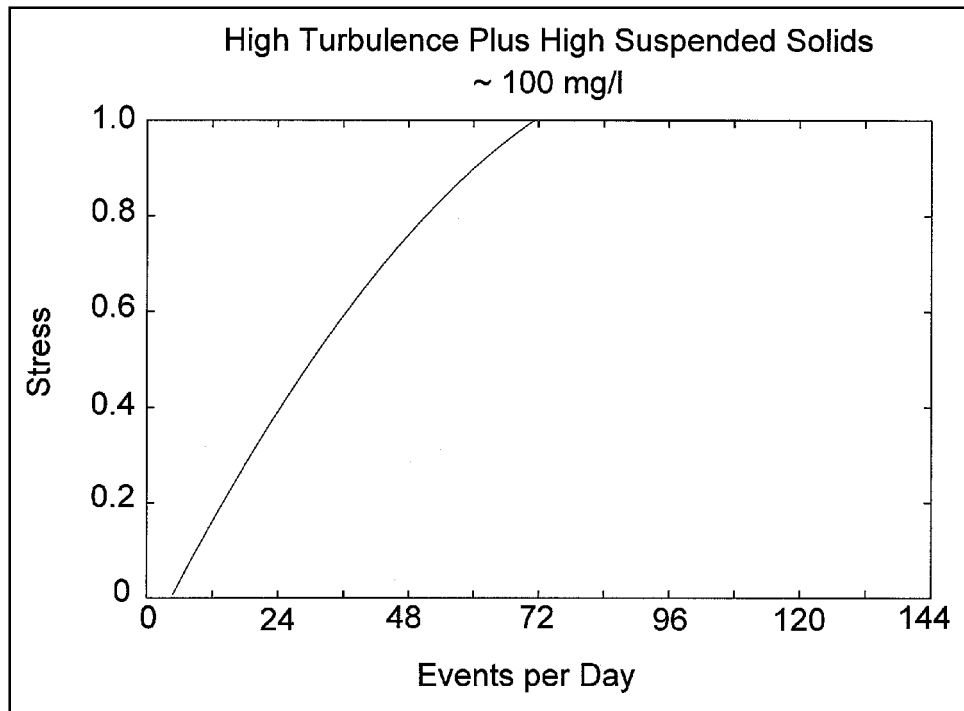


Figure 3. Effects of high turbulence plus high suspended solids on freshwater mussels

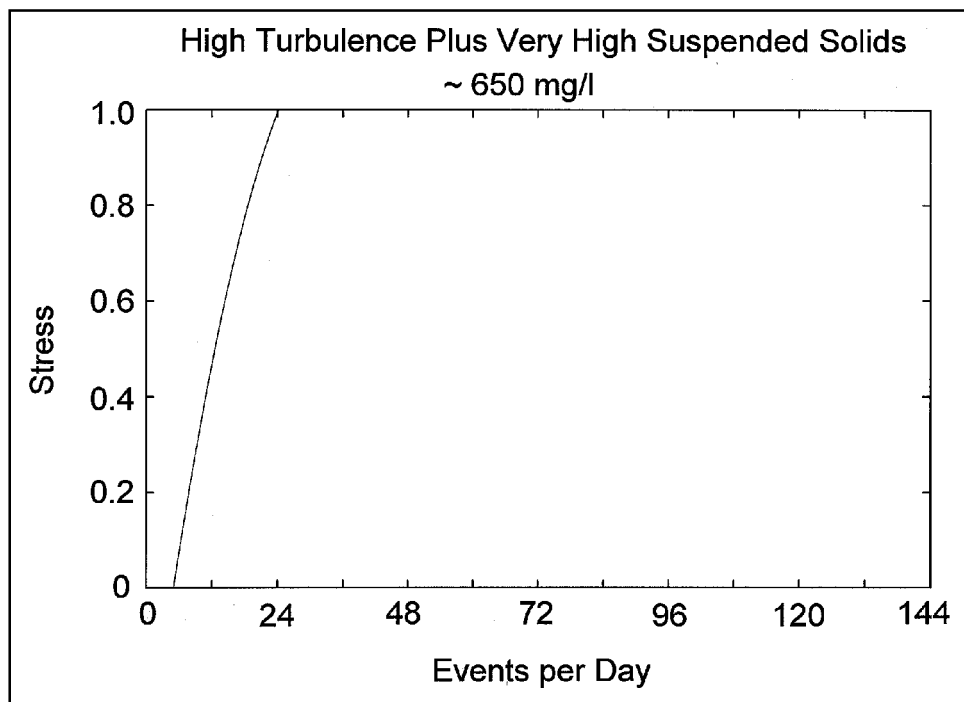


Figure 4. Effects of high turbulence plus very high suspended solids on freshwater mussels

4 Use of a Shell Gape Monitor to Study Effects of Physical Disturbances on Freshwater Mussels

Background

Considerable technological effort is required to continuously monitor flowing water for a chance spill of a toxic substance. However, freshwater mussels can be rigged with a device that will continuously and remotely monitor shell valve movement that indicates ambient water quality (Waller et al. 1994). An early application of this device was to detect toxic discharges using the zebra mussel, *Dreissena polymorpha* (Borcherding 1992). Recently, this device was recommended as a component of monitoring stations of the German Commission for the Protection of the Rhine Against Pollution (Borcherding 1997). Physical disturbances affect freshwater mussels by causing them to temporarily close their valves thereby interfering with normal feeding, respiration, and waste removal.

A bivalve must gape its shells slightly when it pumps water in or out of the mantle cavity. When the two shells are tightly closed, normal feeding and respiration are interrupted. Results of laboratory observations indicate that mussels tightly close their shells when experiencing physical stress. Continuous monitoring of bivalve shell movement can provide an especially meaningful biological record of brief physical disturbances.

Methods

The shell gape monitor consists of four parts: sensors, a signal conditioning control board, an analog to digital (A-D) converter, and a laptop computer and software for data storage, retrieval, and analysis. The sensors produce a magnetic field. A metal object entering that field creates a disruption that is converted by the signal conditioning board to a voltage change. Mussels and sensors are affixed to a plexiglass platform such that the valve farthest from the sensor is stationary and the near valve moves toward the sensor as the mussel opens (Figure 5). A metal disc attached to the moving valve of the mussel is detected by the sensor. Voltage from the signal conditioning board is directly proportional to

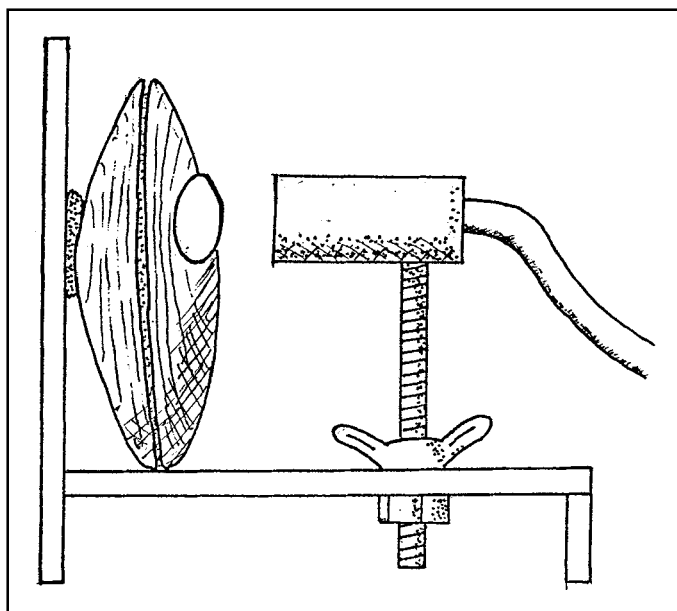


Figure 5. Side view of single sensor directed toward a mussel

the distance between the metal disc and the sensor over a range of approximately 8-14 mm (Figure 6). The sensor is adjustably mounted on the plexiglass platform such that the metal-disc-to-sensor distance can be set at approximately 14 mm when the mussel is tightly closed. Voltage input to the A-D converter is digitized at a rate of 10 samples per second and sent to the laptop computer.

After recording, data are viewed as waveforms, using proprietary software, that can be scaled such that peak voltage represents maximum shell opening and minimum voltage represents tight shell closure. Thus all comparisons are made on a scale

of percent shell gape of an individual mussel. Shape of the waveform, duration of open and closed periods, periodicity, and other aspects of mussel behavior can be characterized.

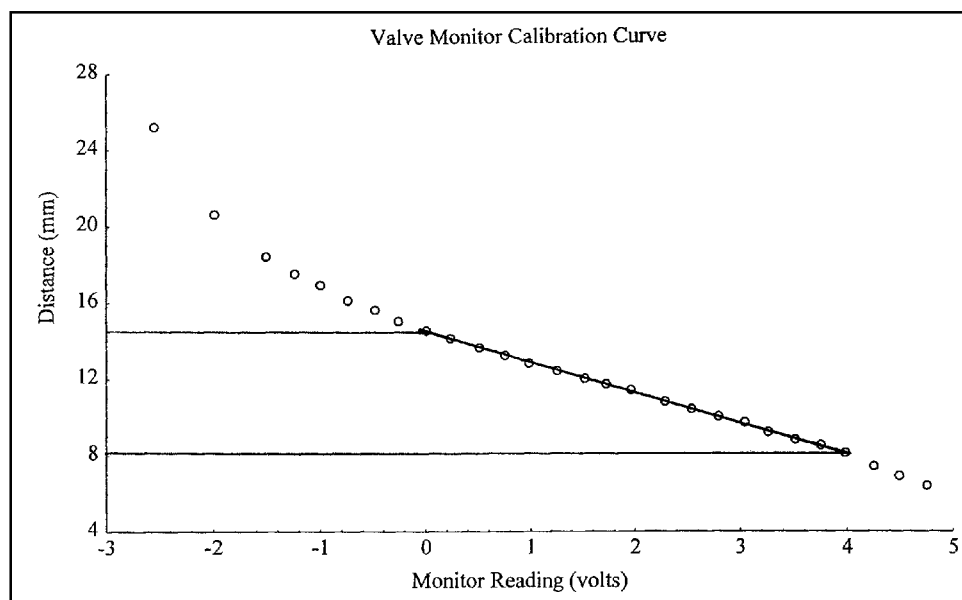


Figure 6. A typical sensor calibration curve showing the linear response from 8 to 14 mm

Results

Preliminary studies

The monitoring device was used to examine the effects of intermittent pulses of high water velocity on behavior of a native unionid, *Amblema p. plicata*. Eight mussels, collected from the Big Sunflower River, Mississippi, were attached to the device, and their behavior was continuously monitored for nine days. Mussels were held in experimental flumes and exposed to a constant water velocity of 11 cm/sec at 14 °C for 3 days to establish baseline behavior. For the next 3 days, mussel responses to intermittent turbulence was monitored by exposing them once every 2 hr to 5-min periods of high water velocity (45 cm/sec). The final 3 days involved a return to continuous exposure to the baseline velocity of 11 cm/sec. A suspension of dried *Chlorella* was added every third day as a food source.

Figure 7a depicts a 4-hr and 42-min period of observation of 8 mussels during the initial 3-day period without turbulence. Figure 7b depicts the same duration of observation during the 3-day period during which the same mussels were exposed to intermittent turbulence. The onset of each 5-min period of turbulence is indicated by a vertical “event mark” that crosses the plots of all 8 mussels. In Figures 7a and 7b, degree of shell gape is indicated by the y-axis against time on the x-axis. Gridlines divide the x-axis into approximately 11-min intervals. Thus, mussel No. 1 was closed for approximately the first 200 min and open for the final 82 min of the observation period depicted in Figure 7a.

Most mussels spent less than half of the duration of the experiment open. Behavior varied considerably among individuals when they were open. Some mussels were very active, with shell gape rhythmically varying between 100 and approximately 50 percent of maximum gape (e.g., mussel No. 6 in Figures 7a and 7b). Other mussels were much less active, with the shell gape staying at approximately 100 percent almost continuously (e.g., mussel No. 7 in Figures 7a and 7b). Neither percent time spent open nor general characteristics of activity changed appreciably during the 3 days of intermittent exposure to turbulence in the middle of the 9-day observation period. Mussels that were open at the onset of a high-velocity pulse typically responded by partially closing their shell valves, with recovery requiring less than 20 min. No mussels responded to intermittent turbulence by fully closing their shell valves. Only 38 percent of all turbulence onset-mussel interactions involved an open mussel.

Field studies in the east channel of the Mississippi River at Prairie du Chien

In August 1996 the shell gape monitor was taken to the east channel of the upper Mississippi River near Prairie du Chien, Wisconsin, for field testing. The apparatus was used to monitor shell gape behavior of 12 specimens of *A. p. plicata*, the threeridge mussel. Because of technical difficulties, continuous data on shell gape behavior were obtained for only 6 mussels. Divers equipped with surface-supplied air were used to deploy and retrieve the apparatus. Data on water velocity perpendicular and parallel to shore were obtained with a Marsh

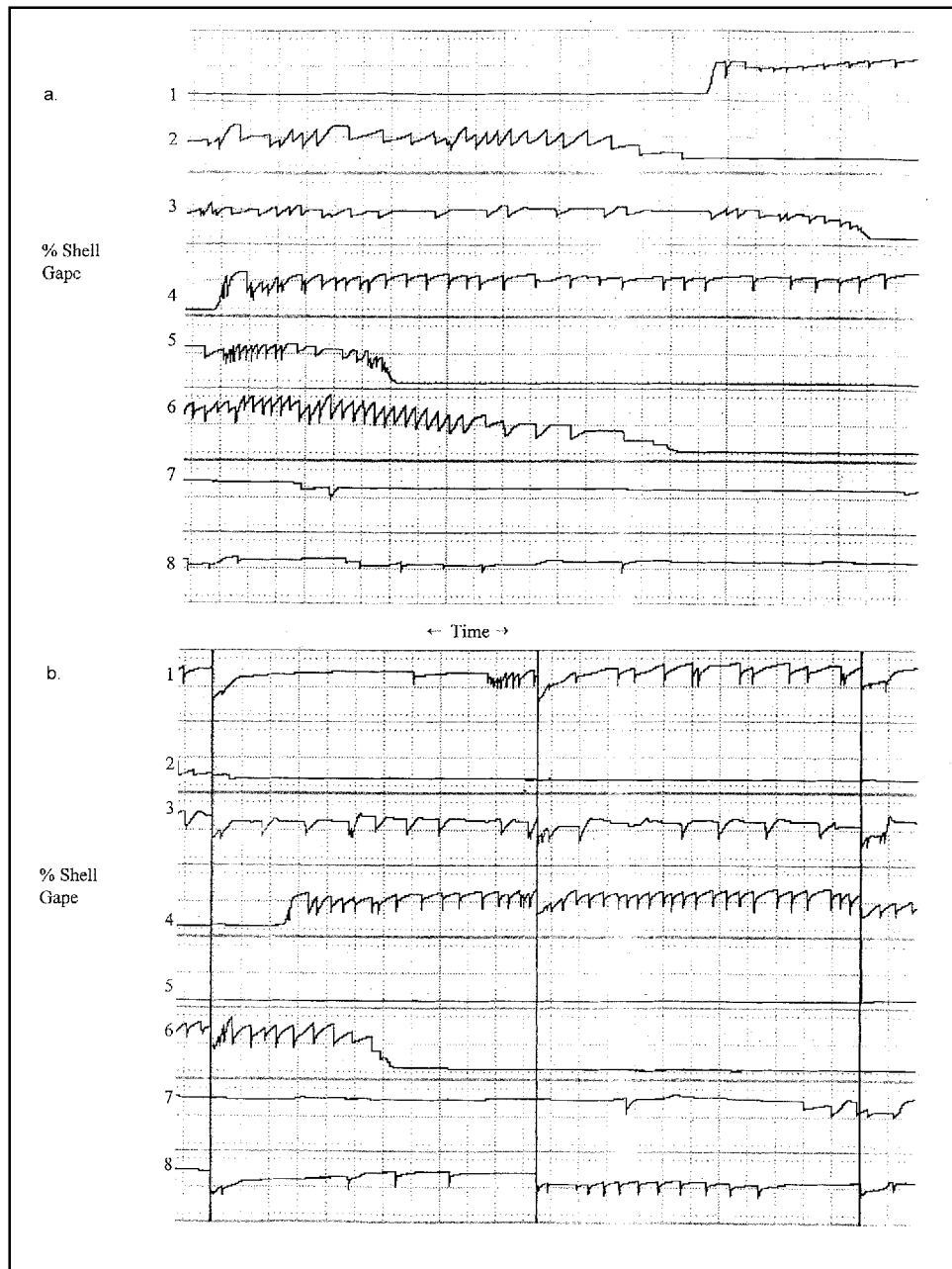


Figure 7. Valve movement patterns of eight mussels exposed to (a) continuous water velocity of 11 cm/sec (upper eight curves) and (b) 5-min pulses of 45 cm/sec water velocity once every 2 hr (lower eight curves)

McBurney 201 current velocity meter. Data on mussel behavior and water velocity were saved to data loggers and returned to the laboratory for analysis and plotting. The purpose was to obtain background data on shell gape behavior during passage of small boats, during passage of commercial vessels, and when no traffic was present.

During a 2-day period without traffic, 5 of the 6 mussels closed their valves for approximately 6 hr during the night (Figure 8). On the second night, 5 of the 6 mussels were closed for varying lengths of time, although behavior lacked the synchrony exhibited during the first night. During the entire 2-day period, valve gape typically varied from 100 to at least 50 percent open. There was no complete closure except during the previously described period during the night. As illustrated in Figure 8, there was an extreme amount of background noise in the data. Although synchrony is apparent, starting and stopping time for valve closure varies among individuals by many minutes. In addition, all individuals appeared to regularly open and shut their valves. The sensor failed on individual No. 6 at midnight of the second day.

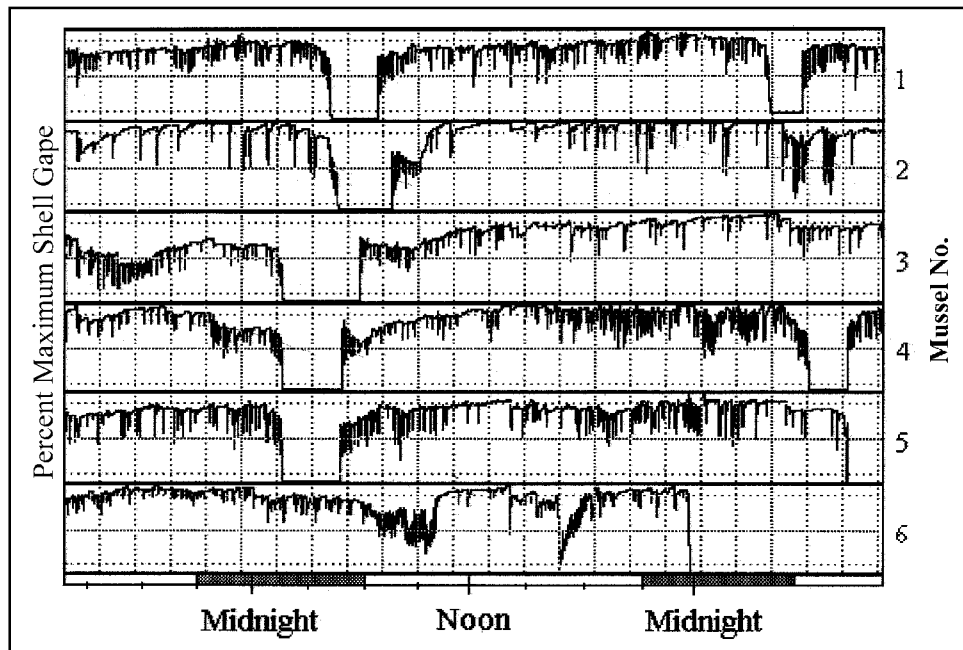


Figure 8. Valve activity of 6 *Amblema p. plicata* over a 2-day period in the east channel of the upper Mississippi River near Prairie du Chien, Wisconsin. (The y-axis is scaled from 0 (closed) to 100 percent of maximum gape for each individual. The shaded bar represents the approximate period of darkness)

Figure 9 displays the results of 60 min of an extremely high amount of pleasure craft activity resulting from a water ski exhibition. Shell gape behavior 60 min before and 60 min after the water ski exhibition are labeled on the figure and separated by a single vertical line. On average, mussels were opened for a significantly longer period of time after the exhibition was over than during the disturbance. The mean gape during the exhibition was 86.6 percent, and after the exhibition it was 89.6 percent. The *t* value was equal to 2.753, with a *P* value of 0.02 (one failed test), with 5 deg of freedom. Figure 9 shows considerable variation among test organisms but a greater number of interruptions in gape when the disturbance took place.

Effects on mussel behavior of a disturbance caused by a 21-ft boat with twin 140-hp outboard motors and the work boat *Gold Cup* (an 800-hp work boat used

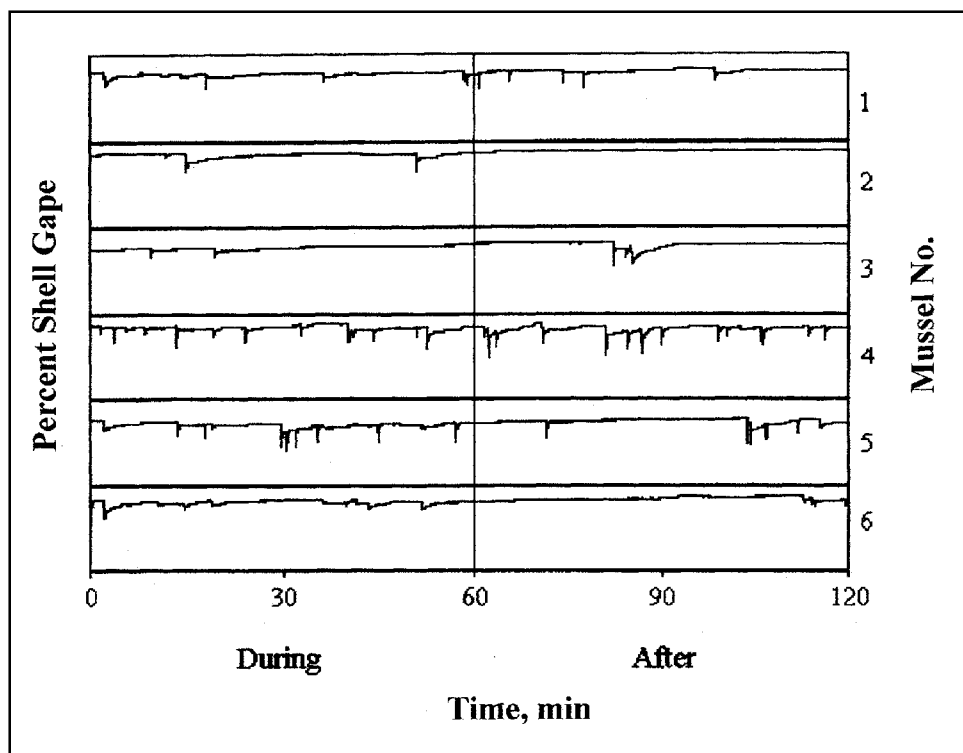


Figure 9. Valve activity of 6 *Amblema p. plicata* over a 2-day period. The first 60 min, to the left of the vertical bar, was a period of high water turbulence brought about by heavy pleasure boat traffic

to move 1-2 grain barges in the east channel of the upper Mississippi River) are displayed in Figure 10. The vertical lines represent disturbance caused by passage of the *Gold Cup* or the 21-ft boat. A total of 7 periods of disturbance, caused by vessel passage, on mussels are displayed in Figure 10. Event No. 1 was caused by upbound passage of the *Gold Cup* pushing a loaded barge. Effects of vessel passage on ambient velocity are shown in Figure 11. Events 2-5 were created by upbound passage of the 21-ft boat. Effects on ambient water velocity are shown in Figures 12 (Event 3), 13 (Event 4), 14 (Event 5), and 15 (Event 6). Water velocity changes caused by downbound passage of the *Gold Cup* without a barge are displayed in Figure 16. Velocity data for Event 2 were lost.

Passage of the *Gold Cup* with a barge caused velocity parallel to flow to increase from approximately 0.6 - 0.8 ft/sec to slightly more than 1.0 ft/sec (Event 1, Figure 11). Mussels 1, 2, 3, and 6 closed briefly during passage. Mussel No. 5 closed for a short period of time several minutes after passage. Mussel No. 4 appeared to be unaffected by vessel passage.

Events 2-6, although resulting from passage of a smaller, noncommercial craft, created measurable effects on ambient water velocity. Considerable variation in response characterized mussels during the remainder of the test. The first test mussel closed briefly during the first two events. During subsequent passages, there appeared to be no relationship between vessel passage and shell gape behavior. Test organism No. 2 remained nearly 100 percent open for the next several hours except for a period of partial closure between events 4 and 5 (see

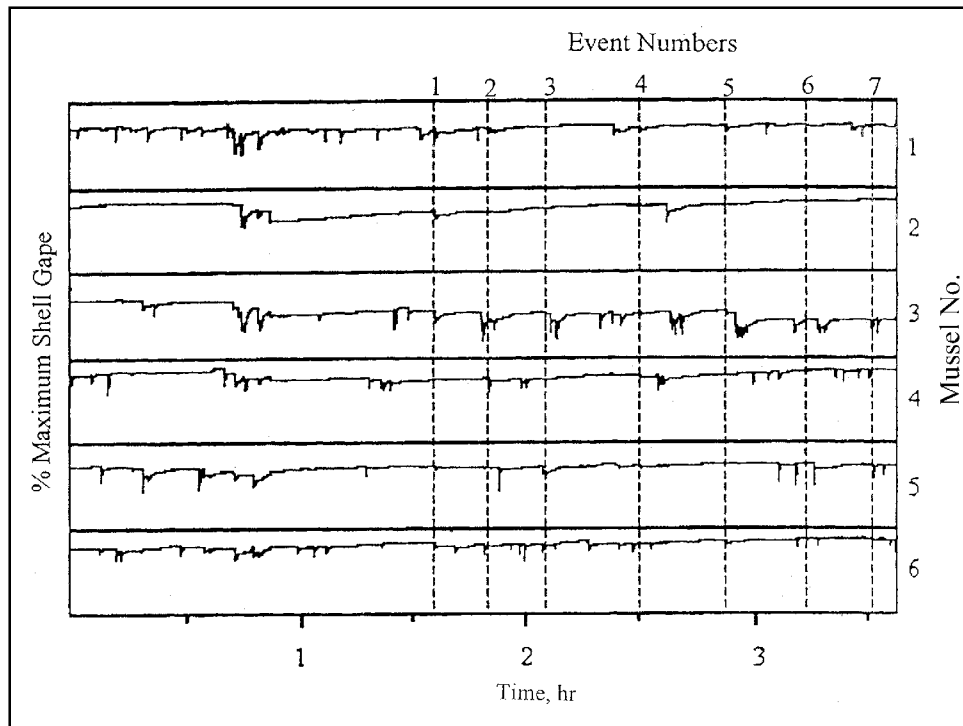


Figure 10. Valve activity of 6 *Amblema p. plicata* over a 2-day period affected by boat passage. The vertical lines represent passage of a 21-ft work boat (Events 1-7, see Figures 11-16)

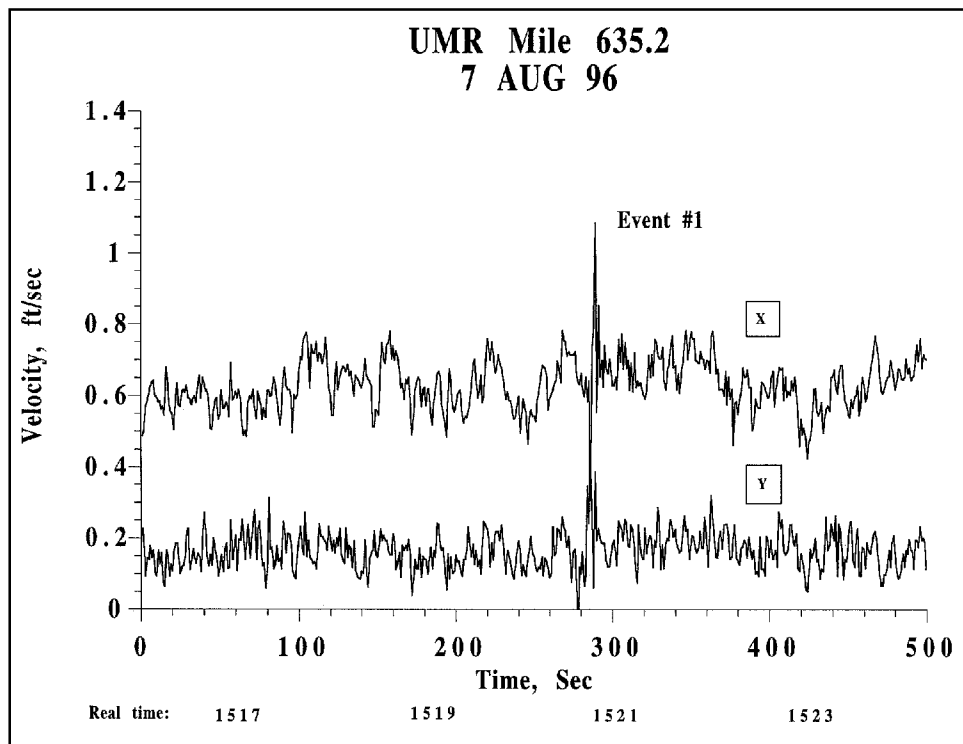


Figure 11. Event 1, upbound passage of the *Gold Cup* with a loaded barge

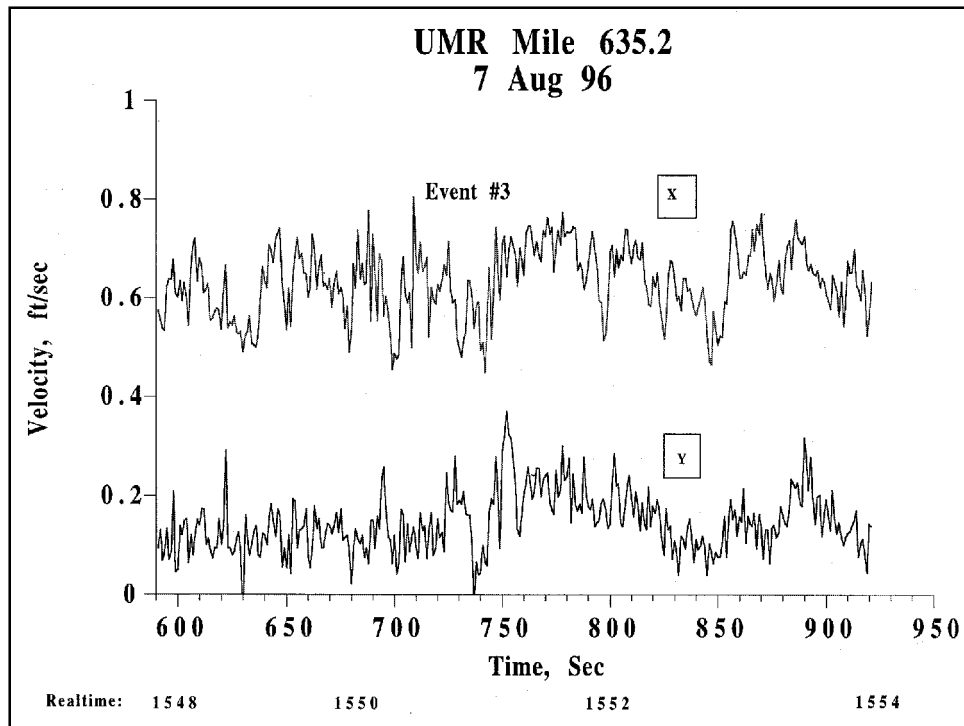


Figure 12. Event 3, downbound passage of a 21-ft boat

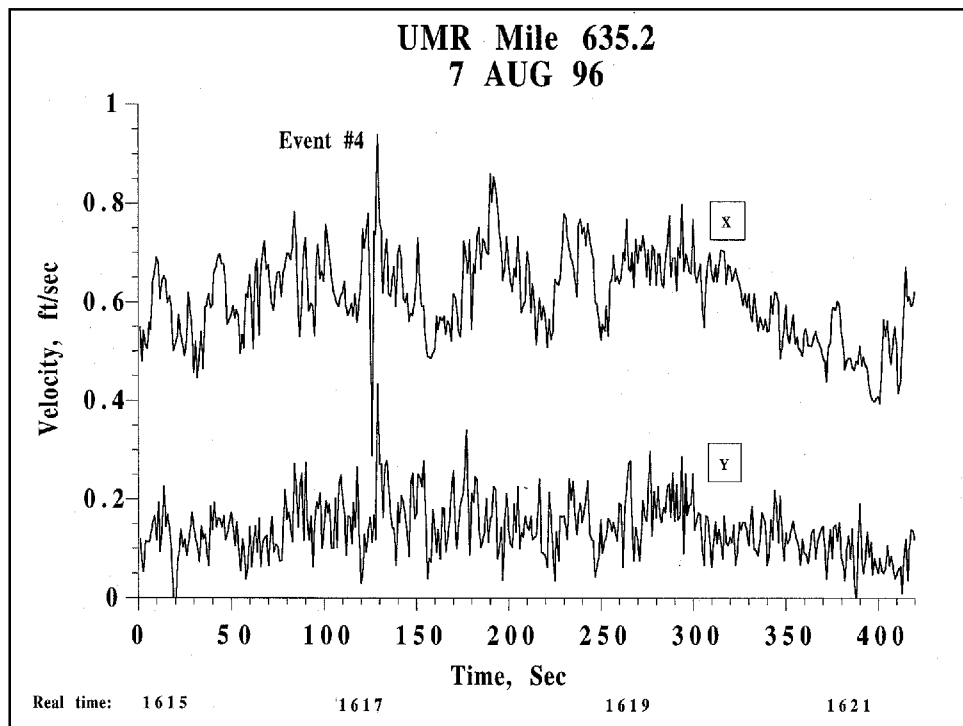


Figure 13. Event 4, downbound passage of a 21-ft boat

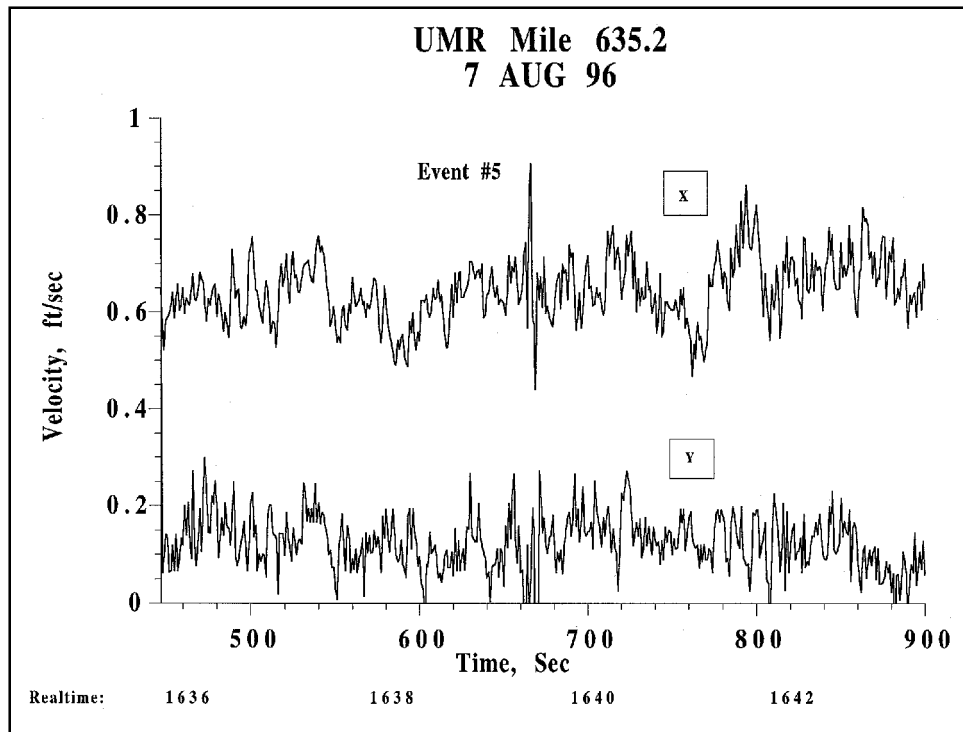


Figure 14. Event 5, downbound passage of a 21-ft boat

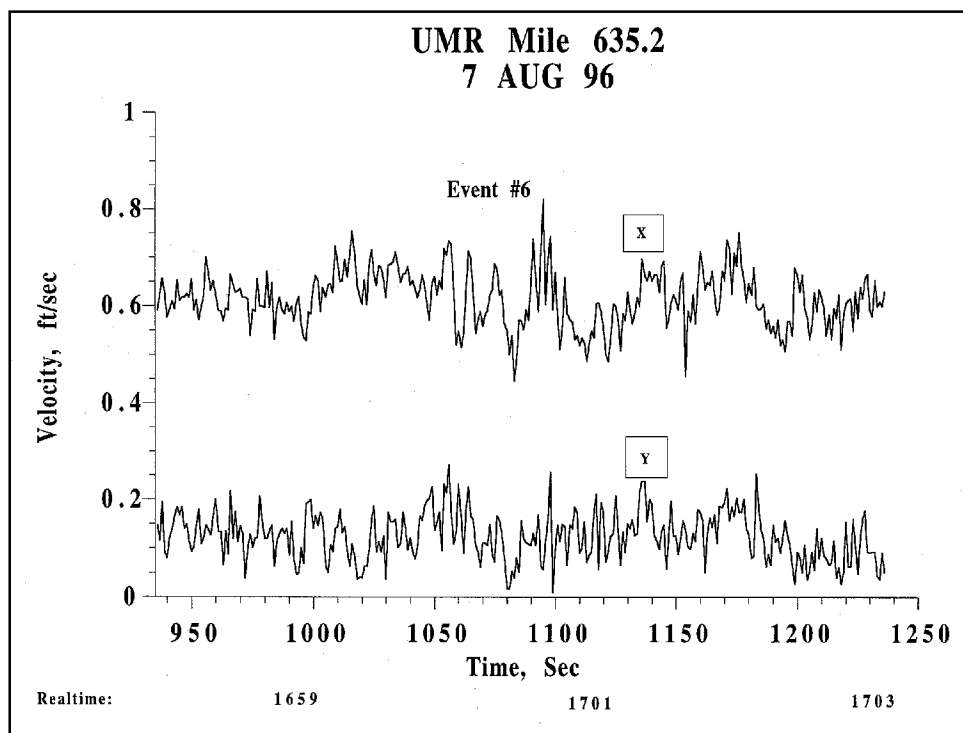


Figure 15. Event 6, downbound passage of a 21-ft boat

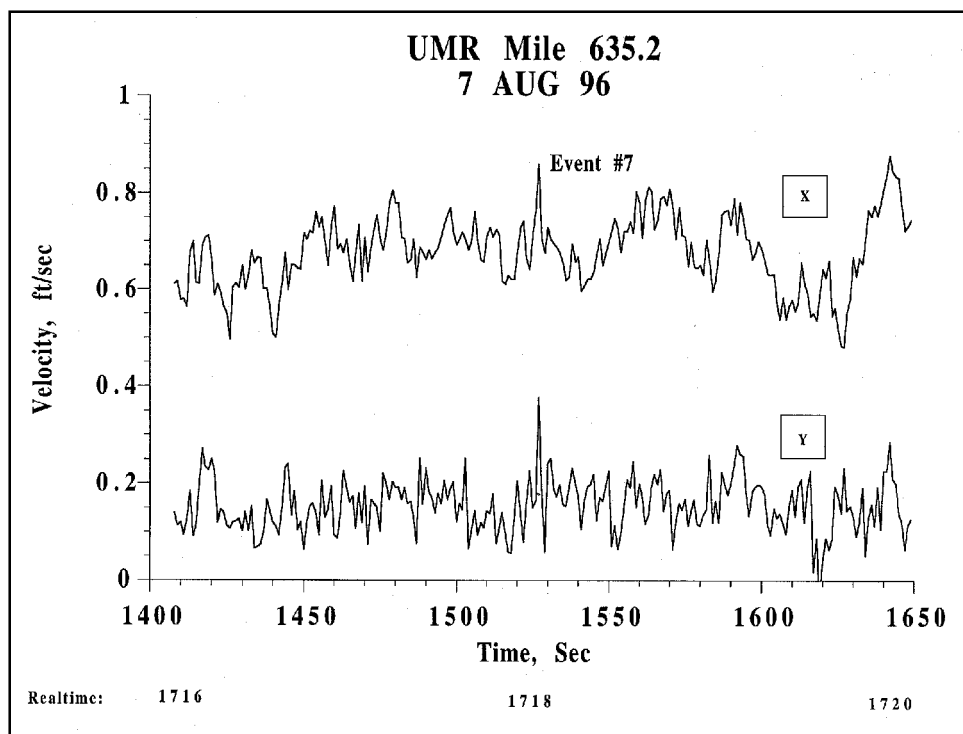


Figure 16. Event 7, downbound passage of the *Gold Cup* without a barge

Figures 13 and 14). Mussel No. 3 exhibited brief closure periods which typically took place after the vessel passed. With the exception of Event 2, behavior of mussel No. 4 appeared unrelated to vessel passage. Mussel No. 5 was unaffected by the first event, but exhibited a response immediately after Event 2 and a minor response during Event 3. It was unaffected by the 4th and 5th passages. It closed down briefly prior to Event 6 (obviously unrelated to vessel passage) and then closed briefly immediately after the last passage. Mussel No. 6 showed brief periods of closure near the time of each vessel passage; however, closures for Events 2, 3, 4, and 6 took place before as well as after vessel passage.

In summary, mussel shell gape behavior was variable among all six test organisms although they were extremely close to one another and presumably all affected by disturbance in a similar manner. It is apparent that under normal conditions mussels are not open continuously. With no disturbance from commercial vessels or pleasure craft, mussels regularly cycle between 100 and 50 percent opened. Periods of closure last 5 min or less and can occur several times an hour. With respect to this test in the upper Mississippi River near Prairie du Chien, these findings indicate that brief periods of disturbance caused by vessel passage do not appear to have a sustained influence on mussel behavior.

5 Other Measures of Stress in Mussels--Fecundity and Condition Analysis

Background

Commercial vessel passage could affect the fecundity (number of fertilized eggs or brooding glochidea) of selected species of freshwater mussels. In freshwater unionids, embryos are brooded in the demibranchs which serve as marsupia. The larvae are highly modified and are released as glochidia which parasitize fish gills to complete their development and to facilitate dispersal. Although the glochidia/fish-host relationship has been well studied, there is little information on the number of glochidia produced. A likely reason for this lack of information is the absence of a reliable method to determine unionid fecundity at all stages of glochidia development. One method is to vigorously stir marsupia that has been preserved in formalin in a known volume of water until the marsupia are broken and the glochidia become evenly distributed in the water. The number of glochidia in an aliquot of the mixture is then counted to determine the fecundity. This method can be applied to marsupia containing mature glochidia that separate freely. It does not work well with earlier stages that develop within egg membranes that adhere together, especially upon fixation, and are difficult to disperse.

A method of determining unionid fecundity that works with all stages of glochidia development would be more useful. Although North America contains the most diverse assemblage of unionids in the world, the continued existence of many species are now either threatened or endangered. An accurate method to measure the fecundity of unionid bivalves would be helpful in determining the effects of environmental quality on their reproduction. The goal of this work was to develop an accurate method of determining the number of glochidia brooded in unionid bivalves. If feasible, this method could be used to assess the effects of various physical disturbances, such as the movement of commercial navigation traffic, on fecundity of freshwater bivalves.

Materials and Methods

Unionid bivalves containing glochidia in various stages of development were collected from the upper Mississippi River near Prairie du Chien, Wisconsin, and the Sunflower River, Mississippi. *Leptodea fragilis*, *Legumia recta*, *Potamilus alatus*, *Obliquaria reflexa*, and *Fusconia flava* were collected from the upper Mississippi River on 31 July and 1 August 1994 whereas *Amblema p. plicata*, *Potamilus dombeyanus*, and *Glebula rotundata* were collected from the Sunflower River on 18 August 1994.

The enzyme proteinase K was used to digest connective tissues holding the glochidia together. Dispersal of the glochidia in the digestion medium was also aided by the presence of SDS (to which proteinase K is tolerant (Ebeling et al. 1974)) in the digestion buffer which helps solubilize proteins. Each mussel was dissected by cutting the anterior and posterior adductor muscle along the inner surface of one of the valves. Swollen gills, indicating the presence of brooding glochidia, were excised for digestion. Each gill was weighed and then digested in a solution containing 100 ug/ml proteinase K, 0.5 percent SDS, 5 mM EDTA, 10 mM Tris-HCl (pH 7.8), and 250 mM NaCl. The samples were incubated at 50 °C until the gills were completely digested so the glochidia could be freely dispersed. The amount of time required for complete digestion varied from 4 to 12 hr. Periodic swirling of the samples greatly aided in accelerating the rate of gill digestion.

Results

The method was successfully used to digest the gills of *Amblema p. plicata*, *Glebula rotundata*, *Leptodea fragilis*, *Legumia recta*, *Potamilus alatus*, and *Plectomerus dombeyanus*. The estimated fecundity of *L. fragilis*, *L. recta*, and *P. alatus* are shown in the following tabulation. *Obliquaria reflexa* and *Fusconia flava* form conglomerates which resisted digestion and thus it was not possible to determine the fecundity of these species using the present method.

Fecundity of Selected Mussel Species			
Species	Mean \pm SE ($\times 10^5$)	Range ($\times 10^5$)	Number
<i>L. recta</i>	2.34 \pm 0.12	2.15 - 2.56	3
<i>L. fragilis</i>	60.78 \pm 8.4	25.62 - 123.54	11
<i>P. alatus</i>	2.67 \pm 0.05	2.62 - 2.72	2

The digestion buffer also served well as a preservative. Digested samples may be stored at room temperature for many weeks for processing at a later time. Digested samples have been stored in the freezer at -20 °C for at least 2 years

without degradation of the sample. Fixatives or preservatives should not be added to the samples. This direct counting method relies on the digestion and solubilization of tissue proteins that are kept in the dissolved state during counting and storage. The addition of fixatives such as formalin will cause the precipitation of organic tissue components and a general mess. The addition of preservatives such as ethanol will do the same. The best way to store the glochidia is in the digestion buffer in the refrigerator or freezer.

In July 1995 *A. p. plicata* were collected from the turning basin and the reference site in the east channel to evaluate the possible effects of traffic movement on fecundity. A total of 43 organisms of approximately the same size were obtained, 20 from the turning basin and 23 from the reference site. Mean fecundity from the turning basin (53.21 ± 9) was not significantly different from that at the reference site (62.1 ± 12 ($p > 0.1$)).

Ratios of dry tissue mass to shell volume and dry shell mass to shell volume were calculated for a subset of the organisms. These data, plus data from three other sites, are shown in the following tabulation. (Sample sizes are variable since some of the tissue data were lost). With respect to both condition indices, there was no significant difference among the sites. Dry tissue was somewhat greater with respect to shell volume in the reference site than in the turning basin. Conversely, dry shell mass, with respect to shell volume, was slightly greater in the turning basin than in the reference site. Neither comparison was significant ($p > 0.1$).

Condition Data on <i>Amblema p. plicata</i> Collected in the UMR near Mile 635 at Prairie du Chien, August 1996						
Site	Dry Tissue/cm³			Dry Shell/cm³		
	mg	SE	Number	mg	SE	Number
Main Channel						
Nearshore	63	7	6	535	13	26
Farshore	93	3	4	512	11	24
East Channel						
Turning Basin	63	1	20	533	13	42
Reference	76	8	7	522	13	27
Sawmill Slough	70	4	7	475	13	27

With respect to the fecundity data, a series of collections would be required to fully answer the question of effects of traffic on fecundity. For example, fecundity at both sites might be similar at the beginning of the season; however, before spawning some of the glochidea at a stressed site could be absorbed, hence

fecundity would be less, although numbers early in the reproductive season might be similar. Since reproductive products are usually produced at the expense of stored tissue, a measured decrease in tissue condition might not necessarily mean that fecundity would be less in a stressed organism. Regardless, neither the physical condition data (tissue mass or shell mass) nor the fecundity data exhibit marked effects of commercial vessel passage.

6 Summary

The lack of substantial physiological response in the present study suggests that mussels were essentially unaffected by treatment conditions. One possible explanation is that even the least stressful treatment condition elicited the maximum observable response. A treatment involving no turbulence and no TSS would have been needed to accept this first possibility. However, such extremely benign conditions are unrealistic with respect to these mussel species in large inland waterways. A second possibility is that none of the treatment conditions exceeded a response threshold. However, more stressful treatments would have exceeded conditions that have been measured at mussel beds and thus were not part of the present study design.

Studies by Aldridge, Payne, and Miller (1987) clearly indicate that physiological responses observed in the present study were much less than maximum sublethal responses that might have been elicited. Furthermore, if maximum sublethal responses characterized mussels in all treatments of the present study, it is curious that no mortality was observed. Aldridge, Payne, and Miller (1987) used much more stressful treatments than those of the present study. In turn, their study elicited clear and substantial physiological shifts. The more stressful experimental conditions they used demonstrated that the physiological assessments made herein are a useful method for assessing sublethal effects of intermittent turbulence and TSS. However, the much more stressful conditions used by Aldridge, Payne, and Miller (1987) are irrelevant to the physical conditions proximal to mussels beds in the river at present. More representative conditions used in the present study elicited no substantial physiological response. These treatment conditions were based on a maximum frequency in the upper Mississippi River of one measurable event every 3.3 hr and a TSS rise from an ambient condition of 20 mg/L to 120 mg/L. These conditions accurately represent navigation-induced physical disturbances in the upper Mississippi River.

The results of this (or any) laboratory study cannot be directly extrapolated to the field. Nonetheless, these laboratory results should be considered in light of the sustained abundance of healthy communities of mussels in the upper Mississippi River and lower Ohio River, some in close proximity to navigation channels. With respect to mussels, and after both laboratory and field studies are considered, it is appropriate to conclude that intermittent physical effects of routine commercial navigation traffic do not warrant great concern.

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13. ABSTRACT (Maximum 200 words) Commercial navigation traffic in large inland waterways can cause brief episodes of increased turbulence and suspended solids, both of which are potentially disruptive to essentially sessile, filter-feeding mussels. Predicting the consequences of traffic to mussels is difficult due to the intermittent, brief nature of changed physical conditions. Previous laboratory studies indicated that aspects of physiological energetics, including filtration rate, respiration rate, nitrogen excretion rate, O:N ratio, and tissue condition index, are sensitive indicators of physiological stress of traffic on mussels. Previous studies, using very high suspended solids concentrations and frequencies of disruption, showed an additive effect of increased suspended solids to turbulence and provided evidence that the frequency of intermittent disturbance was important. In short-term experiments, upward shifts in O:N by mussels in the most severely stressed treatment groups proved to be the best indicators of shifts toward a negative bioenergetic balance. In longer-term studies of turbulence effects, mussels under the most severe stress (continuous high turbulence) showed reduced tissue-to-shell mass ratios. <div style="text-align: right;">(Continued)</div>				
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In the present study, turbulence effects were investigated in an experiment long enough to elicit such tissue condition index changes, using an array of frequencies of exposure treatments that spanned the range likely to be encountered by mussels in the upper Mississippi River. Frequency of intermittent exposure to high-turbulence levels had no relationship to condition changes in terms of filtration rate, respiration rate, nitrogen excretion rate, O:N, or tissue condition index. Additional short-term laboratory experiments were conducted to investigate additive effects of suspended solids to turbulence, using frequencies of exposure and levels of suspended solids much more realistic than those of the previous studies. Evidence of an additive effect of suspended solids was more equivocal than in the harsher experiments of the previous studies. Physiological disruption was slightly greater when high suspended-solids concentration accompanied intermittent turbulence. The tendency was for downward shifts in nitrogen excretion and upward shifts in O:N. However, this tendency was not manifest in all species within an experiment nor among experiments for particular species. Although some statistically significant shifts were measured, major changes in metabolic condition generally were not indicated. No changes in tissue condition occurred. Studies of shell valve gape behavior indicated that mussels sometimes responded to navigation traffic effects by slightly closing their shell for a brief period. However, such behavior is varied substantially among mussels and for an individual over time.

In general, physical habitat disruption associated with routine navigation traffic tends to elicit minor shifts upward in O:N and measurable changes in shell gape behavior. These are relatively subtle physiological responses, consistent with the subtlety of brief, infrequent episodes of turbulence and elevated TSS. Although such responses can be elicited and measured, their biological significance appears to be slight. Results of all laboratory experiments have been summarized in a series of curves which relate potential level of stress to a mussel versus the four possible effects of commercial vessel passage: low and high turbulence without suspended solids, high turbulence plus high suspended solids, and high turbulence plus very high suspended solids.