A Completion Report on

METABOLIC EFFECTS OF SUSPENDED SOLIDS ON UNIONID MUSSELS

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ABSTRACT

Freshwater unionids are an important ecological and economic resource. Their numbers have been declining at astounding rates and many species have already become extinct. Habitat alterations, especially increased amounts of suspended solids entering water systems, are thought to be a leading cause of mussel declines. The purpose of this research was to determine if high concentrations of suspended solids found commonly in rivers and streams in Indiana interfere with normal metabolism in *Quadrula pustulosa* (Lea 1831), *Amblema plicata* (Say 1817), and *Lampsilis radiata* (Lamark 1819). Metabolic shifts that occur in mussels and other aquatic poikilotherms resulting from decreased uptake and/or assimilation of food and oxygen have served as useful indicators of environmental stress.

Metabolic parameters (oxygen consumption rate, total ammonia nitrogen excreted, food clearance rate, and the oxygen to nitrogen ratio) were measured in individual mussels under controlled laboratory conditions before and after exposure to suspended solids and under field conditions after exposure to ambient concentrations of suspended solids in Little Pine Creek. Our results do not support the conclusions of Aldridge et al. (1987). Significant differences in physiological parameters were not detected in *Q. pustulosa* and *A. plicata* after exposure to 500 and 1000 mg/l bentonite clay when compared to controls. However, physiological parameters changed significantly over time, suggesting an overall decline in health of mussels maintained under control and treatment conditions. In support of this conclusion, significant differences in physiological parameters were found between field collected and laboratory held populations of *Q. pustulosa* and *L. radiata*. Oxygen to nitrogen ratios were significantly greater in laboratory held mussels while the other physiological parameters were generally less in the laboratory held individuals when compared to field collected individuals. Oxygen consumption and food clearance rates, which were the most reliable parameters in *L. radiata*, were significantly lower in individuals of *L. radiata* maintained at a first order region of Little Pine Creek than in individuals maintained at a third order region of the stream, suggesting a difference in

condition caused by water quality. The upstream site was higher in suspended solids and nutrients, but lower in temperature during that time.

INTRODUCTION

Bivalves (Phylum Mollusca; Class Bivalvia) are important in many freshwater communities and often account for the greatest biomass (Russell-Hunter et al. 1983). The most ecologically significant family of freshwater bivalves, the Unionidae, is the only group associated with large, permanent drainages. Unionids are widely distributed despite the obligate parasitic stage in their life cycle (Burky 1983); five hundred of the approximately one thousand species of unionids were once found throughout the United States (Isom and Hudson 1982). However, pollution and other habitat disturbances that alter flow rates and increase turbidity levels in aquatic ecosystems have led to the decline and extirpation of many species (Fuller 1977). Extinctions of many mussel species have already occurred in Indiana and other states and many species remain characterized as threatened or endangered (Cummings and Berlocher 1990). High levels of suspended solids have been identified by the Indiana Department of Natural Resources as a likely threat to the survival of the endangered white cat's paw pearly mussel (*Epioblasma obliquata perobliqua*) and other unionids (IN State Project No. E-1-5). Dineen (1971) found only half of the mussel species in St. John's River, Indiana that were recorded by Wenninger (1921) and van der Schalie (1936). The Tippecanoe River in Indiana still has a rich diversity of mussel species, but of the 34 species of unionids found there (Cummings and Berlocher 1990), nine are endangered in Indiana. Because the annual recruitment in freshwater mussel populations is typically low and sporadic (Neves and Widlak 1987), information regarding mussel health and habitat requirements will be essential for their protection.

High levels of silt in the water disrupts the life cycle by destroying habitat for adults and juveniles and by decreasing the survival of host fish that disperse juvenile mussels (Fuller 1977). Young mussels are particularly sensitive to silt which clogs their gills (Negus 1966). It is assumed that high levels of suspended solids cause adult unionids to reduce respiration and feeding activity which would consequently decrease their growth and reproduction. The resulting decreased scope for growth (an estimation of an organism's available energy for growth and reproduction) reduces fecundity at least indirectly (Bayne et al. 1981). Moreover, silt and dissolved organic matter act synergistically with metallic pollution to decrease bivalve survival by interfering with shell calcification (Rosenberg and Henschen 1986).

Populations of the approximately fifty species of mussels along the Tennessee River and its tributaries have been severely impacted by increased suspended sediment levels resulting from industrial and hydropower operations (Stansbery and Clench 1974; Dennis 1981). The declining mussel diversity in the Mississippi River is also believed to be due to increased siltation (Havlik and Stansberry 1977). The Red River in Kentucky once supported a wide diversity of mussel populations but accelerated sedimentation has eliminated many species (Bradfield and Porter 1990).

Suspended solids, defined as the organic and inorganic particulate matter in water (EPA 44015-86-001), are a serious problem in many aquatic ecosystems. Poor land practices involving irrigation, mining, dredging, hydropower production, and construction activities often substantially increase concentrations of suspended solids in aquatic systems. In addition, commercial navigation traffic causes short term but often severe turbidity, turbulence, and wave wash (Miller et al. 1987).

Suspended solids adversely affect the feeding process in some marine molluscs by reducing the rate and efficiency of feeding (Moore 1977). The rate of water transport in unionids is greatly affected by the amount of suspended matter in the water (Jorgensen 1955). Bivalves generally respond to high siltation levels by partially or completely closing their valves. Extended periods of valve closure result in decreased oxygen uptake and assimilation, leaving the animal in a hypoxic or anoxic state. Badman (1974) has shown that the filtration rate in hypoxic unionids is reduced even when the siphons are extended and apparently filtering.

Respiratory measurements often indicate how an organism is responding to environmental conditions and can provide a quantitative measure of how rapidly energy and oxygen are used. Of the two methods of measuring metabolic rate, direct and indirect calorimetry, the indirect measure, or the oxygen consumption rate, is the most sensitive for aquatic poikilotherms (Chech 1990).

Bivalves are often found in environments with fluctuating oxygen concentrations, and some species have evolved strategies to endure short term oxygen poor conditions. Metabolic responses to anoxic conditions vary within and especially between species. Anodonta cygnea survived twenty-two days of anaerobic conditions (Zs.-Nagy et al. 1982). The metabolic rate usually decreases initially but if poor conditions persist, the metabolism will become partially or fully anaerobic, which may sustain the mussel until oxygen becomes available (Giese 1969). However, long term anoxic conditions prevent mussels from building up energy reserves necessary for reproduction to sustain the population.

The anaerobic pathways used by bivalves simultaneously degrade glycogen and aspartate to the endproducts alanine and succinate. Succinate may be further degraded into volatile fatty acids such as propionate or acetate if anaerobic metabolism persists (de Zwaan 1983; Zs.-Nagy et al. 1982). During anoxic conditions, most energy is derived from the catabolism of carbohydrates (the Pasteur effect) (De Zwaan and Wijsman 1976). When carbohydrate reserves are depleted, bivalves catabolize remaining reserves of protein and/or lipid (Gabbot 1976). Anaerobic pathways employed by bivalves produce 4.71 to 6.43 moles of ATP per mole of glucose and are thus more efficient than the glycolytic pathway in mammals which yields 2 moles of ATP per mole of glucose catabolized (deZwaan 1983). ATP production under anaerobic conditions is still significantly reduced and organic acids may accumulate or be excreted in greater amounts than under aerobic conditions (De Zwaan and Wijsman 1976). However, bivalves mobilize calcium carbonate from the shell to buffer respiratory acidosis (Hemming et al. 1988).

Because glycogen is the major substrate for anaerobic metabolism, the fall accumulation of glycogen stores may help protect mussels from anoxia during winter ice cover. Likewise, summer-conditioned glycogen poor mussels are less tolerant of anoxia (Holopainen 1987). The sizes of stored energy reserves vary greatly with season; lipid stores usually range from 1.5 to 10% dry weight but may exceed carbohydrate reserves during certain times of the year, depending on factors such as food availability and reproductive condition (Dare 1973). *Annodonta cygnea* is composed of 5 to 35% glycogen (DeZwaan and Wijsman 1976). *Mytilus edulis* (Class Bivalvia; Family Mytilidae) changes from reliance on carbohydrates in the summer to proteins in the winter (Bayne 1972).

The metabolic strategies employed under different conditions are extremely important to the survival of the organism and to the recruitment rate of mussel populations. Bayne (1975) has shown that gametogenesis represents a significant proportion of total energy expenditure of bivalves and that during periods of stress, *Mytilis edulis* catabolizes proteins, carbohydrates, and lipids. In addition, larvae spawned from individuals under temperature and nutritive stress had lower survival rates (Bayne 1972). Helm et al. (1973) found that larvae spawned from stressed adults had different biochemical compositions and slower growth rates than larvae spawned from adults in normal conditions.

Nutritive and other types of stress in aquatic invertebrates has been monitored by the measurement of oxygen taken up and metabolic wastes excreted. Stickle (1971) and Russell-Hunter and Eversole (1976) have measured catabolic substrates used within and between species, during different seasons, reproductive states, and environmental conditions. Metabolic shifts that occur in mussels and other aquatic poikilotherms resulting from decreased

uptake and/or assimilation of food are measured by the proportionate amounts of nitrogen excreted and oxygen consumed (Aldridge et al. 1987; Russell-Hunter et al. 1983; Ikeda 1977; Widdows 1978; Stickle 1971; Emerson 1967). These measurements have been shown to be useful indicators of environmental stress in molluscs (Widdows 1978; Bayne et al. 1979). Aldridge et al. (1987) used this technique on three unionid mussels to demonstrate their reliance on nonprotein reserves when suspended solids levels were continuously high (600-750 ppm). Naimo et al. 1992 also found increased oxygen : nitrogen ratios in *Lampsilis ventricosa* after exposure to 111 and 305 µg/l cadmium. Similarly, Ikeda (1977) observed the shift from a protein based metabolism to a carbohydrate and lipid oriented metabolism (high oxygen : nitrogen and oxygen : phosphorus ratios were measured) in starved zooplankton.

Unionids are an index of water pollution because they are extremely sensitive to changes in water and habitat quality and are usually associated with unpolluted ecosystems (Fuller 1977). Substrate composition and current velocity are two important habitat characteristics affecting mussel populations (Gordon and Layzer 1989). In lotic environments, unionids prefer shallow areas with slow, steady currents and sandy substrates -- environments low in suspended silt and turbulence but high in suspended food (Salmon and Green 1982). Destruction of these stream characteristics, by damming or channelization, results in the loss of mussel populations. Wilson and Clarke (1912) reported a complete loss of mussels in channelized portions of the Yellow and Kankakee Rivers in Indiana. The thirteen species of mussels still found in the Kankakee River were primarily in regions with coarse substrates and low suspended silt levels (Bringham et al. 1981).

In order to optimally manage ecosystems, environmental agencies need methods and criteria for the measurement of sublethal effects of environmental pollutants on aquatic organisms. At present, state agencies are unable to implement suspended solids standards which protect mussel populations because information concerning harmful concentrations and lengths of exposures is not known. However, acute lethality tests require the destruction of large numbers of animals, cannot be done on endangered species, and do not address long term deleterious effects. It is often useful, but also more difficult, to assess how a pollutant present in the environment at sublethal levels may impair essential functions and consequently threaten a species' survival (Eagle 1981). Optimal indices should be quick, inexpensive, noninvasive, and most importantly, sensitive measures of sublethal responses to stress in organisms.

This study was an attempt to employ a more sensitive indicator of stress than death to assess unionid habitat requirements. The effects of concentrations of suspended solids (found commonly in rivers and streams in Indiana) on metabolism in *Quadrula pustulosa* (Lea 1831),

Amblema plicata (Say 1817), and *Lampsilis radiata* (Lamark 1819) were determined. Experiments with suspended solids (500 ppm and 1000 ppm) were conducted on the Pimpleback (*Quadrula pustulosa*) and the Threeridge (*Amblema plicata*). The Fat Mucket (*Lampsilis radiata*) was translocated from a lake with low ambient levels of suspended solids to a stream with transiently high levels of suspended solids. Oxygen consumption rates (VO_2), total ammonia nitrogen excretion rates (ANE), food clearance rates (FCR), and oxygen to nitrogen ratios (O:N) were used to assess metabolism in the mussels following the approach of Aldridge et al. (1987) and Naimo et al. (1992). With improved understanding of unionid responses to suspended solids and other disturbances, state and federal agencies will be better able to monitor unionid health. The determination of water quality requirements for unionids, which are poorly defined at present, is essential for the development of sound water quality criteria. Nonpoint sources in watersheds may then be managed for improved mussel habitat.

Q. pustulosa and *A. plicata* are tachytictic (short-term) breeders which commonly inhabit large to medium sized rivers. Reproductive individuals are gravid from June to August. *L. radiata* is a bradytictic (long-term) breeder found in slower flowing streams, lakes and impoundments. Reproductive individuals may remain gravid from August to July. These species were chosen for these studies because they are commonly found in habitats with moderate to good water quality and probably represent moderately tolerant species (Cummings and Berlocher 1990).

METHODS

Adults of *Quadrula pustulosa* (Pimpleback) and *Amblema plicata* (Threeridge), collected on 7/13/92 and 8/23/92 from the Tippecanoe River near Battleground, Indiana, and adults of *Lampsilis radiata* (Fat Mucket), collected on 5/14/92 and 5/27/92 from Crooked Lake, Indiana, were maintained at 20°C in flow-through tanks containing unchlorinated well water and aquarium gravel. Species were identified according to Oesch (1984). Mussels were fed a suspension of yeast, algae, and ground trout chow (approximately one tsp each mixed in one liter of water) every other day. An identification number was engraved on the shell of each individual. Mussels used in experiments were maintained in the laboratory prior to experimentation for only a three to five day acclimation period. Because mussel health, measured by the tissue condition index or the relative flesh weight to shell weight, deteriorates rapidly under artificial conditions (Naimo et al. 1992), fresh mussels were collected from the field no more than five days before experimentation began. All of the mussels used in the experiment appeared to be healthy (they did not gape and filtered normally). Furthermore, initial metabolic measurements in these mussels were similar to initial measurements recorded in unionids by others (Aldridge et al. 1987 and Naimo et al. 1992). Other individuals of *Q. pustulosa* and *L. radiata* were maintained under these laboratory conditions for ten months.

Measurement of Physiological Condition under Control and Experimental Conditions

All metabolic measurements (oxygen consumption, ammonia nitrogen excretion, and food clearance rates) were made at a constant temperature of 20° C and low light levels since both temperature (Lewis 1984) and light (McCorkle et al. 1979) have a significant effect on unionid respiration. *L. radiata* was the only sexually dimorphic species used in this study and even in this species, it was impossible to identify gravid individuals without dissection. This should not have been a problem, however, because metabolic measurements did not differ consistently between males and females of *L. radiata*. Measurements were based on the dry weight of each mussel, determined after experimentation, where individual mussel soft tissues were dried to a constant weight for two days at 75°C. Because Bauer et al. (1991) could not base oxygen consumption measurements on the dry weights of the endangered species, *Margaritifera*

margaritifera and *Unio crassus*, they estimated dry weights based on the relationship between length and weight of empty shells. An attempt was made to predict the flesh weight of *L. radiata* from a regression of wet weight (y) in grams and length of empty shells (x) in millimeters ($y = 59.78 + 0.55x$; $r^2 = 0.796$) (Figure A7). The regression did not predict flesh weights accurately enough to be used for calculating metabolic parameters and was not used in these experiments. Wet and dry weights for the three species used in these experiments are in tables A1 to A6.

Oxygen Consumption and Total Ammonia Nitrogen Excretion Rates

The catabolic substrate of each individual under conditions free of suspended solids was determined by measuring the total ammonia nitrogen excretion rate (ANE in µmol N/g*l*h) and oxygen consumption rate (VO_2 in µmol O_2/g*l*h). To measure oxygen consumption rates, individual mussels were placed in a closed container with 80 to 100% oxygen saturated water and initial and final dissolved oxygen readings were taken. Three chambers of different sizes (473ml, 940ml, 1400 ml) were used depending on the size of the mussel. Dissolved oxygen was measured with a Yellow Springs Instrument (YSI) Model 50B Meter. The amount of dissolved oxygen remaining in the chamber at the end of the metabolic measurement was never less than 5.4 mg/l. To measure concentrations of ammonia nitrogen excreted, mussels were placed in 200 ml of oxygenated well water for one hour (larger mussels were placed in 500 ml). Total excreted ammonia accounts for nearly all of the excreted nitrogen in molluscs (Russell-Hunter 1983) and was measured spectrophotometrically by the phenate method (American Public Health Association 1975). Ammonia nitrogen concentrations (y) in mg/l were calculated from absorbance at 630 nm (x) with the calibration equation: $y = -0.0103 + 0.00031x$; $r^2 = 0.98$. Repeated nitrogen excretion and oxygen consumption rate measurements were taken on laboratory cultured individuals of *Q. pustulosa* and *L. radiata* to determine how consistent these measurements are in individuals over time (Table A8). Mean variation in oxygen consumption rates in individuals over time was 3.77 µmol O_2/l*h for *Q. pustulosa* and 2.03 µmol O_2/l*h for *L. radiata*. Mean variation in nitrogen excretion rates in individuals over time was 0.64 µmol N/l*h for *Q. pustulosa* and 0.86 µmol N/l*h for *L. radiata*.

Oxygen to Nitrogen Ratios

Ratios of oxygen consumed to nitrogen excreted (O:N), derived from the ANE and VO_2 rate determinations for each individual, are a measure of the proportion of protein catabolized by the mussel (Ikeda 1977; Russell-Hunter et al. 1983; and Aldridge et al. 1987). O:N ratios of less

than 30 indicate protein based catabolism (Bayne and Widdows 1978). O:N ratios in aerobic mussels under control conditions should therefore be approximately 30 or less, while mussels forced into anaerobic conditions by high suspended solids or other stressors would be expected to have greater O:N ratios.

Food Clearance Rates

The food clearance rate was used as a relative estimate of the feeding rate under standard conditions. Food clearance rates were determined by measuring the amount of suspended yeast removed from a known concentration of yeast suspension in water. Yeast concentrations were measured spectrophotometrically at 550 nm and recorded as mg yeast/g*l*h using a calibration curve ($y = -0.00002 + 0.035x$; $r^2 = 1.0$). Each mussel was placed in 200 ml of oxygenated well water with 8 mg yeast/100 ml of water for at least 30 minutes. Larger individuals were placed in 500 ml of water with the same concentrated yeast suspension.

Laboratory Exposures to Suspended Solids

Metabolic changes over time in mussels exposed to suspended solids under standard laboratory conditions were compared to metabolic changes in mussels maintained under control conditions. Experimental units consisted of 38-liter aquaria filled with 26 liters of gently aerated well water (20°C) and environmentally realistic concentrations (500 and 1000 ppm) of bentonite clay suspended by recirculating pumps. Bentonite clay comprises approximately 20% of Wabash drainage basin soils (D.Norton, pers comm.). The average size of bentonite clay particles is 2 μm. The laboratory light cycle (16L:8D) was consistent with the natural photoperiod. Thirty individuals of *Q. pustulosa* and twelve individuals of *A. plicata* were randomly divided into four groups of approximately equal size distribution. Half of the mussels of each species were maintained in the suspended solids treatment (fed their normal ration) for two weeks while the others (controls) were maintained under identical conditions in water without suspended solids. To prevent build up ammonia, the water was changed weekly in the aquaria while the metabolic measurements were being taken. Metabolic activity in individuals was recorded before the start of the experiment and on days 7 and 14 by measuring the oxygen removed, total ammonia nitrogen excreted, and yeast particles cleared from suspension. After the two week exposure period, all of the mussels were maintained in water without suspended solids while all other conditions remained the same. A previous study measured metabolic changes in unionids after one week of exposure to suspended solids and turbulence (Aldridge et al. 1987). Exposure periods in this study were longer so that any effect seen after one week of exposure could be confirmed after the second week. A change in response would indicate adaptation to the conditions. The one week recovery period was

employed to determine whether the effects of the treatment were long term (causing damage to the mussels) or short acting. Individuals were removed only briefly from the treatment to make these physiological measurements. First, individuals in one of the four groups were placed in a respiration chamber. After oxygen consumption rates were measured, the mussels were returned to their treatment while respiratory measurements were made on mussels in the other groups. Next, ammonia nitrogen excretion rates were measured on individuals in the same order, and finally, food clearance rates were measured in a similar manner. Thus, after each metabolic measurement, mussels were returned to their treatment or control conditions. After the one week recovery period, the same physiological measurements were taken on the mussels. All measurements were made at 20°C.

Physiological Measurements under Ambient Suspended Solids Levels

Fourty-eight individuals of *L. radiata* were collected from Crooked Lake (Noble and Whitley County) on 5/14/92 and on 5/27/92; half were transported to the Purdue Animal Science Farm stream gage station (Tippecanoe County) located at the headwaters of Little Pine Creek and half to the Greenhill gage station (Warren County) where the stream is third order. Long-term data on stream discharge, suspended solids, nitrate, total phosphorus, conductivity, pH, and temperature are available for this stream. The mussels were held inside the gage stations in flowing water (3.8 L per minute) from the creek, so that levels of suspended solids and other water quality characteristics were identical to ambient levels. Metabolic activity was measured initially, after rain events, and during low flow conditions. Mussels were maintained at the downstream site (Greenhill gage station) from 5/25/92 to 6/25/92 and at the upstream site (Animal Science Farm gage station) from 6/3/92 to 6/23/92.

Statistical Analysis

The general linear models (GLM) procedure in the Statistical Analysis System (SAS) was used for all analysis of variance (ANOVA) tests. Variances were normally distributed and no significant differences were found between replicates in each treatment in the laboratory experiments. The replicates were pooled and analyzed by repeated measure analysis. Therefore, metabolic changes in individuals after one, two, and three weeks were compared to the initial metabolic measurements of those individuals with respect to treatment.

Physiological data from the upstream and downstream sites were analyzed separately by a two-tailed paired t-test where individual measurements over time were analyzed. Data from the two

stations were compared by a two tailed t-test. Physiological measurements taken on laboratory cultured and on freshly collected mussels were also analyzed by a two-tailed t-test.

RESULTS

Controlled Laboratory Exposures to Suspended Solids

Initial metabolic measurements were taken on both groups of the thirty individuals of *Q. pustulosa* after a three day acclimation period to laboratory conditions before the experiments with 500 ppm and 1000 ppm bentonite clay. These initial measurements are the most accurate estimations of normal, unstressed metabolism in mussels. However, the four metabolic parameters were significantly different between the group of *Q. pustulosa* collected in July and the group collected in August (Tables 1 and 2). Mean oxygen consumption rates ranged from 8.52 to 17.66 μmol O_2/g*l*h, with the greater rates in the group collected in August. Ammonia nitrogen excretion was greater in the group collected in July (mean rates were 1.43 and 1.30 μmol N/g*l*h) than in the group collected in August (mean rates were 0.50 and 0.49 μmol N/g*l*h). Oxygen to nitrogen ratios were less in the group collected in July (mean ratios were 8.12 and 7.47) than in the group collected in August (mean ratios were 39.93 and 32.77). Finally, food clearance rates were greater in the group collected in July (mean rates were 1.56 and 2.30 mg yeast/g*l*h) than in the group collected in August (mean rates were 0.68 and 0.92 mg yeast/g*l*h). Possible explanations for these differences are discussed later when these two groups are compared to a group maintained under laboratory conditions.

Initial measurements were also taken on the two groups of twelve individuals of *A. plicata* collected at the same times (July and August) as the groups of *Q. pustulosa*. As with *Q. pustulosa*, there were differences in some of the physiological parameters between the two groups. Standard errors were larger in the physiological measurements taken in *A. plicata* due to the smaller sample sizes used in those experiments. Physiological parameters (oxygen consumption rates, ammonia nitrogen excretion rates, oxygen to nitrogen ratios, and food clearance rates) differed not only between the two groups but within the group collected in August as well. The randomly selected control group in the experiment with 1000 ppm bentonite clay had significantly lower oxygen consumption rates and food clearance rates, and higher oxygen to nitrogen ratios throughout the experiment. Even though initial measurements may have differed between groups, these experiments were designed to measure predicted changes in physiological parameters (decreased oxygen consumption and nitrogen excretion

rates, increased oxygen to nitrogen ratios, and decreased food clearance rates) after exposure to suspended solids.

Quadrula pustulosa Exposure to 500 ppm Bentonite Clay

Oxygen consumption rates appeared to decline in both the treatment and control during the course of the experiment (Figure 1) although initial rates were not significantly higher than those at two weeks or during recovery. Ammonia nitrogen excretion rates (Figure 1) in the suspended solid treatment increased over control rates after the second week of exposure ($p<.05$) but decreased to control levels after the one week recovery period. Oxygen to nitrogen ratios (Figure 1) increased slightly over time in both the treatment and control but remained below 30. Food clearance rates (Figure 1) did not change over the exposure or recovery time or between treatments (Table 1).

Quadrula pustulosa Exposure to 1000 ppm Bentonite Clay

As in the previous experiment with 500 ppm bentonite clay, oxygen consumption rates (Figure 2) seemed to decline over time but did not differ between the suspended solids treatment and the control. Ammonia nitrogen excretion rates (Figure 2) increased after the first week in both the treatment and control but decreased subsequently in both groups. Oxygen to nitrogen ratios (Figure 2) did not differ between the treatment and control and did not increase until the one week recovery period. Food clearance rates (Figure 2) were greater in the control group after one week ($p<.05$) but were not different from the treatment group after the second week of exposure or after the recovery period (Table 2).

Amblema plicata Exposure to 500 ppm Bentonite Clay

Oxygen consumption rates (Figure 3) declined after one and two weeks of exposure, however, the control group responses were not different from the treatment group responses. Ammonia nitrogen excretion (Figure 3) did not differ over exposure time in the treatment or between treatments. Oxygen to nitrogen ratios (Figure 3) increased over time but there were no significant differences between treatments or over time. Food clearance rates (Figure 3) decreased after one week of exposure ($p<.05$) and after two weeks of exposure ($p<.05$) in the suspended solids treatment. After the one week recovery period, food clearance rates returned to control levels (Table 3).

Figure 1. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20°C for *Quadrula pustulosa* before, during, and after exposure to 500 ppm bentonite clay.

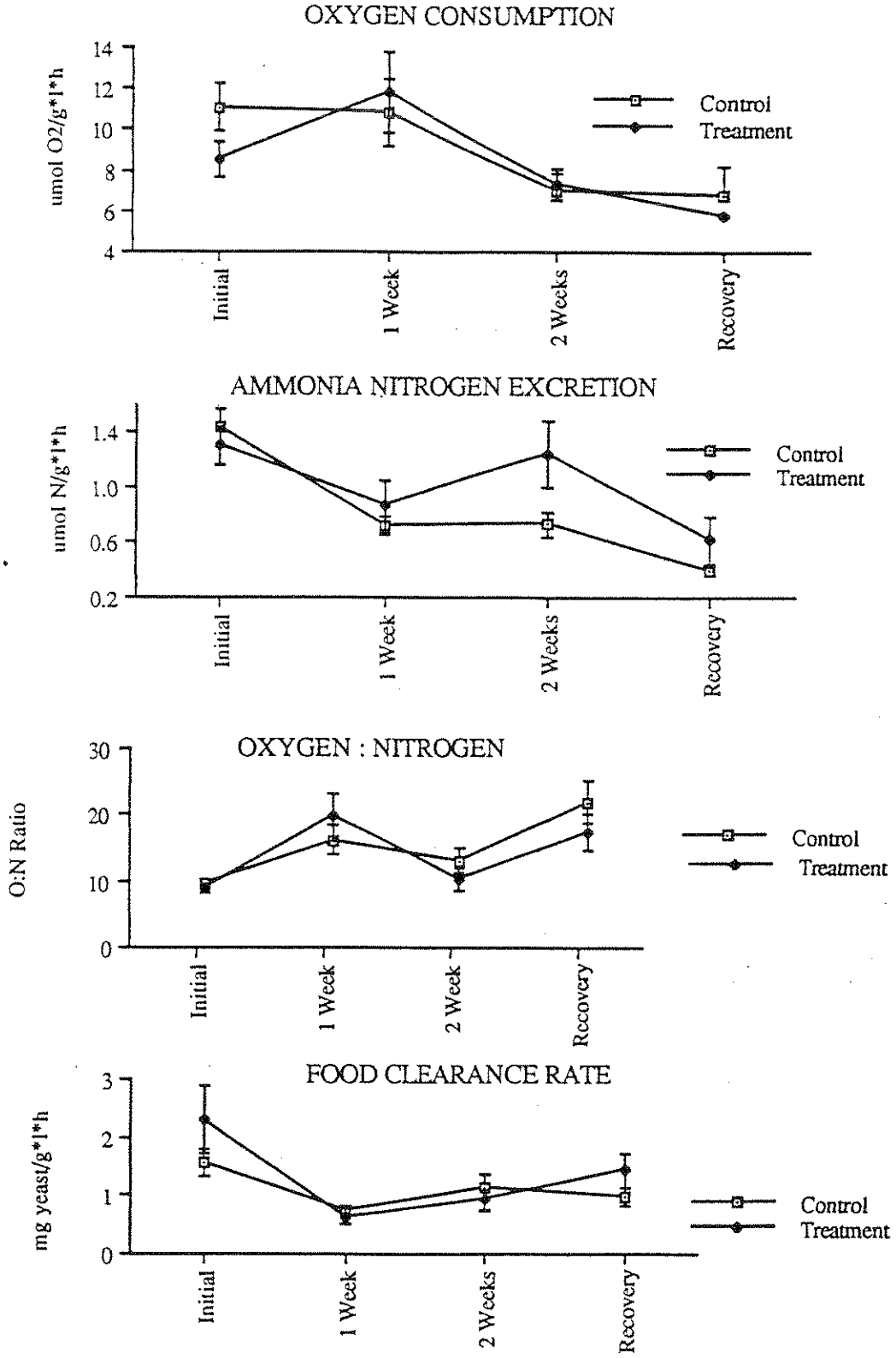

OXYGEN CONSUMPTION
umol O2/g*1*h
14
12
10
8
6
4
Control
Treatment
Initial
1 Week
2 Weeks
Recovery
AMMONIA NITROGEN EXCRETION
umol N/g*1*h
1.4
1.0
0.6
0.2
Control
Treatment
Initial
1 Week
2 Weeks
Recovery
OXYGEN : NITROGEN
O:N Ratio
30
20
10
0
Control
Treatment
Initial
1 Week
2 Week
Recovery
FOOD CLEARANCE RATE
mg yeast/g*1*h
3
2
1
0
Control
Treatment
Initial
1 Week
2 Weeks
Recovery

Figure 1.

Table 1. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20° C for *Quadrula pustulosa* before, during, and after exposure to 500 ppm bentonite clay. A p value of .05 or less shows a difference between treatments when compared to initial measurements and n=15 per treatment.

Oxygen Consumption (μmol O_2/g*l*h)	Control	Treatment	p
Initial	11.08 (1.17)	8.52 (0.84)	
1 Week Exposure	10.82 (1.61)	11.79 (1.99)	.69
2 Weeks Exposure	6.94 (0.89)	7.30 (0.79)	.73
1 Week Recovery	6.75 (1.38)	5.80 (0.78)	.56
Ammonia Nitrogen Excretion (μmol N/g*l*h)	**Control**	**Treatment**	**p**
Initial	1.43 (0.14)	1.30 (0.14)	
1 Week Exposure	0.72 (0.06)	0.86 (0.19)	.47
2 Weeks Exposure	0.73 (0.09)	1.24 (0.24)	.01
1 Week Recovery	0.39 (0.06)	0.62 (0.16)	.04
Oxygen to Nitrogen Ratio	**Control**	**Treatment**	**p**
Initial	8.12 (0.64)	7.47 (0.78)	
1 Week Exposure	14.73 (2.17)	18.28 (3.53)	.43
2 Week Exposure	11.75 (2.03)	8.91 (1.66)	.25
1 Week Recovery	20.55 (3.27)	16.12 (2.67)	.23
Food Clearance Rate (mg yeast/g*l*h)	**Control**	**Treatment**	**p**
Initial	1.56 (0.24)	2.30 (0.59)	
1 Week Exposure	0.73 (0.09)	0.64 (0.14)	.41
2 Weeks Exposure	1.14 (0.21)	0.92 (0.18)	.39
1 Week Recovery	0.96 (0.16)	1.43 (0.29)	.19

Figure 2. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20°C for *Quadrula pustulosa* before, during, and after exposure to 1000 ppm bentonite clay.

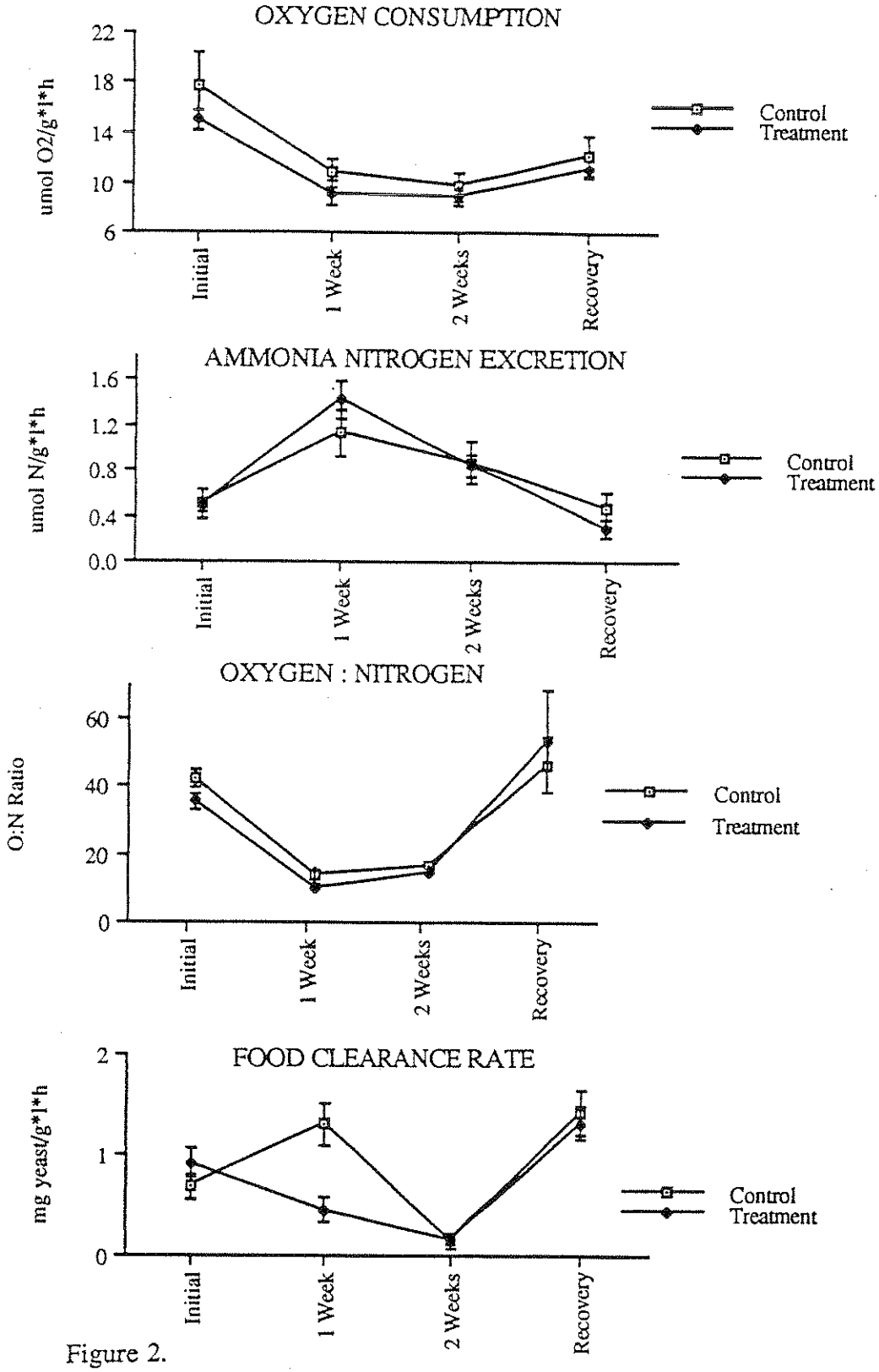

OXYGEN CONSUMPTION
umol O2/g*l*h
22
18
14
10
6
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
AMMONIA NITROGEN EXCRETION
umol N/g*l*h
1.6
1.2
0.8
0.4
0.0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
OXYGEN : NITROGEN
O:N Ratio
60
40
20
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
FOOD CLEARANCE RATE
mg yeast/g*l*h
2
1
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment

Figure 2.

Table 2. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20° C for *Quadrula pustulosa* before, during, and after exposure to 1000 ppm bentonite clay. A p value of .05 or less shows a difference between treatments when compared to initial measurements and n=15 per treatment.

Oxygen Consumption (µmol O_2/g*l*h)	Control	Treatment	p
Initial	17.66 (2.66)	15.02 (0.76)	
1 Week Exposure	10.74 (1.17)	9.04 (0.99)	.57
2 Weeks Exposure	9.69 (1.13)	8.85 (0.67)	.85
1 Week Recovery	12.26 (1.57)	11.20 (0.74)	.65
Ammonia Nitrogen Excretion (µmol N/g*l*h)	**Control**	**Treatment**	**p**
Initial	0.50 (0.12)	0.49 (0.05)	
1 Week Exposure	1.13 (0.21)	1.42 (0.17)	.16
2 Weeks Exposure	0.87 (0.18)	0.84 (0.09)	.89
1 Week Recovery	0.46 (0.14)	0.30 (0.08)	.19
Oxygen to Nitrogen Ratio	**Control**	**Treatment**	**p**
Initial	39.93 (2.63)	32.77 (2.45)	
1 Week Exposure	11.45 (1.21)	7.39 (0.98)	.16
2 Weeks Exposure	13.55 (1.21)	11.59 (1.10)	.64
1 Week Recovery	43.91 (7.95)	50.87 (15.05)	.77
Food Clearance Rate (mg yeast/g*l*h)	**Control**	**Treatment**	**p**
Initial	0.68 (0.13)	0.92 (0.14)	
1 Week Exposure	1.30 (0.22)	0.45 (0.12)	.01
2 Weeks Exposure	0.15 (.02)	0.15 (0.08)	.98
1 Week Recovery	1.42 (0.23)	1.32 (0.16)	.30

Figure 3. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20°C for *Amblema plicata* before, during, and after exposure to 500 ppm bentonite clay.

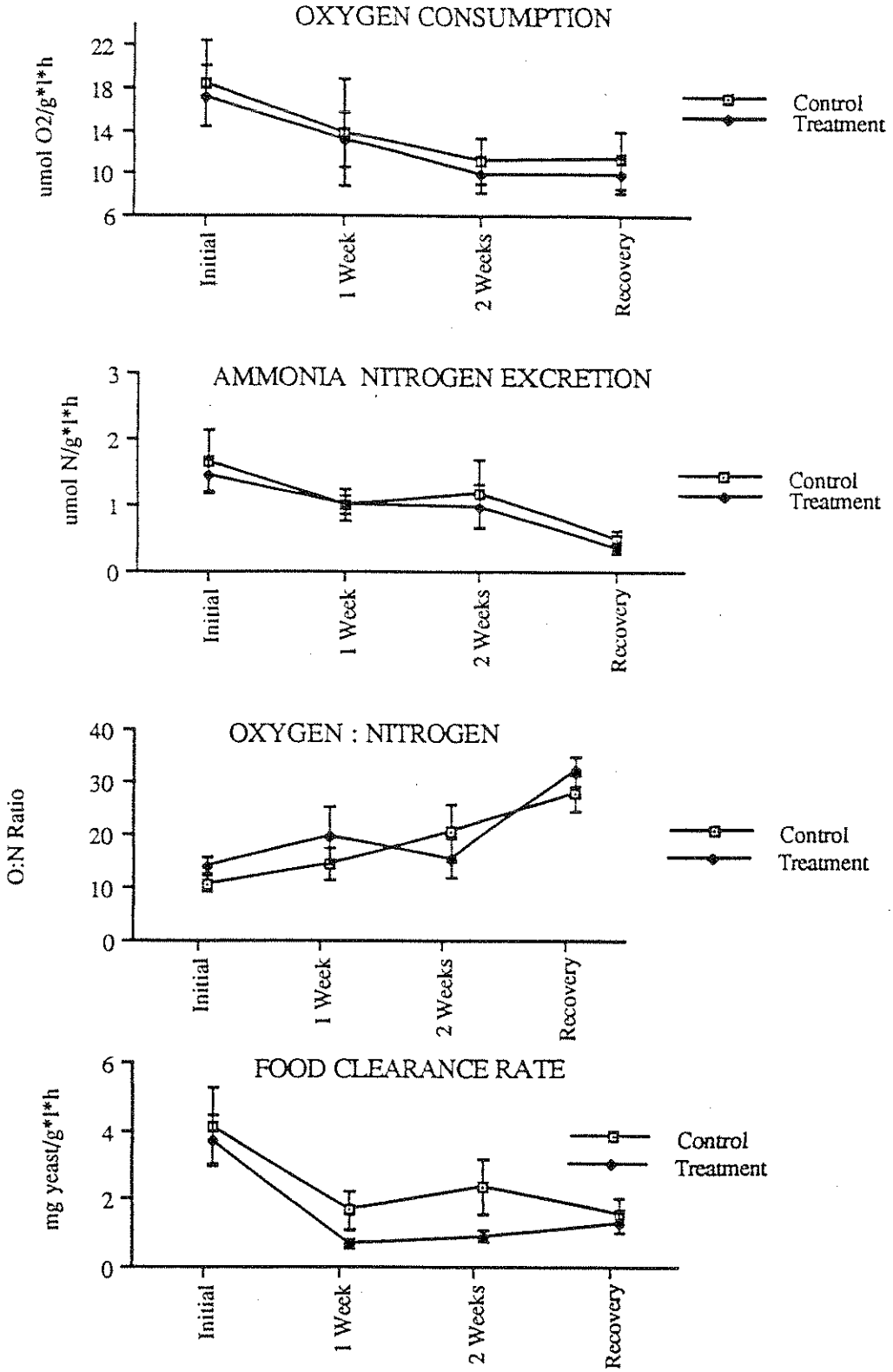

OXYGEN CONSUMPTION
umol O2/g*l*h
22
18
14
10
6
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
AMMONIA NITROGEN EXCRETION
umol N/g*l*h
3
2
1
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
OXYGEN : NITROGEN
O:N Ratio
40
30
20
10
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
FOOD CLEARANCE RATE
mg yeast/g*l*h
6
4
2
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment

Figure 3.

Table 3. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20°C for *Amblema plicata* before, during, and after exposure to 500 ppm bentonite clay. A p value of .05 or less shows a difference between treatments when compared to initial measurements and n=6 per treatment.

Oxygen Consumption (μmol O_2/g*l*h)	Control	Treatment	p
Initial	18.38 (4.08)	17.24 (2.82)	
1 week exposure	13.77 (5.05)	13.12 (2.60)	.91
2 weeks exposure	11.20 (2.12)	9.80 (1.72)	.27
1 week recovery	11.28 (2.62)	9.88 (1.64)	.09
Ammonia Nitrogen Excretion (μmol N/g*l*h)	**Control**	**Treatment**	**p**
Initial	1.67 (0.48)	1.44 (0.26)	
1 week exposure	0.99 (0.14)	0.99 (0.24)	.99
2 weeks exposure	1.18 (0.52)	0.98 (0.32)	.75
1 week recovery	0.49 (0.13)	0.36 (0.08)	.38
Oxygen to Nitrogen Ratio	**Control**	**Treatment**	**p**
Initial	8.80 (1.53)	12.34 (1.53)	
1 week exposure	12.65 (3.04)	17.93 (5.48)	.38
2 weeks exposure	18.50 (5.27)	13.63 (3.84)	.44
1 week recovery	25.98 (3.47)	30.29 (2.73)	.41
Food Clearance Rate (mg yeast/g*l*h)	**Control**	**Treatment**	**p**
Initial	3.87 (1.12)	3.44 (0.74)	
1 week exposure	1.39 (0.58)	0.38 (0.13)	.02
2 weeks exposure	2.09 (0.82)	0.64 (0.17)	.01
1 week recovery	1.25 (0.49)	1.02 (0.30)	.83

Amblema plicata Exposure to 1000 ppm Bentonite Clay

Oxygen consumption rates (Figure 4) remained the same between the treatment and control groups during the first week of exposure, but were greater in the suspended solids treatment group ($p<.05$) after the second week of exposure and after the recovery period ($p<.05$). Ammonia nitrogen excretion rates (Figure 4) and oxygen to nitrogen ratios (Figure 4) were not different between the two groups or over time. Food clearance rates (Figure 4) were not different throughout the exposure period but after the one week recovery period, the treatment group had a greater food clearance rate ($p<.05$) than the control group (Table 4).

Ambient Exposures of Suspended Solids in *Lampsilis radiata* at the Upstream Site

Figure A2 shows the suspended solids concentrations during, the year before, and several months after the ambient exposure experiments. `There were no large or moderate rain events while the mussels were at the gage station located at Purdue University's Animal Science Farm (6/3/92 to 6/23/92). Physiological measurements were taken on the individuals after one week, when suspended solids averaged 77 ppm and after two weeks, when ambient suspended solids averaged 131 ppm. During this time, ambient nitrate concentrations averaged 20 ppm, nitrite concentrations averaged 0.65 ppm, and total phosphate concentrations averaged 0.01 ppm. Weekly average water temperatures ranged from 14.2 to 15.7 ^{o}C.

Oxygen consumption rates (Figure 5) decreased significantly after one week and after two weeks in the stream water as compared to initial control measurements. Ammonia nitrogen excretion rates (Figure 5) decreased significantly after one week but returned to control levels after the second week of exposure. Oxygen to nitrogen ratios (Figure 5) remained in the healthy range (less than 30). Food clearance rates (Figure 5) increased after the two week exposure period (Table 5). Weekly average water temperatures ranged from 14.1 to 24.6 ^{o}C.

Ambient Exposures of Suspended Solids in *Lampsilis radiata* at Downhill Site

There were no large or moderate rain events while mussels were maintained at the gage station located at Greenhill (5/29/92 to 6/25/92). The range of averaged suspended solid concentrations during the mussels' exposure was 2 to 20 ppm with an average of 9 ppm. Nitrate concentrations ranged from 1.99 to 10.0 ppm, nitrite concentrations ranged from 0.12 to 0.3 ppm, and phosphate concentrations averaged 0.06 ppm.

Figure 4. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20°C for *Amblema plicata* before, during, and after exposure to 1000 ppm bentonite clay.

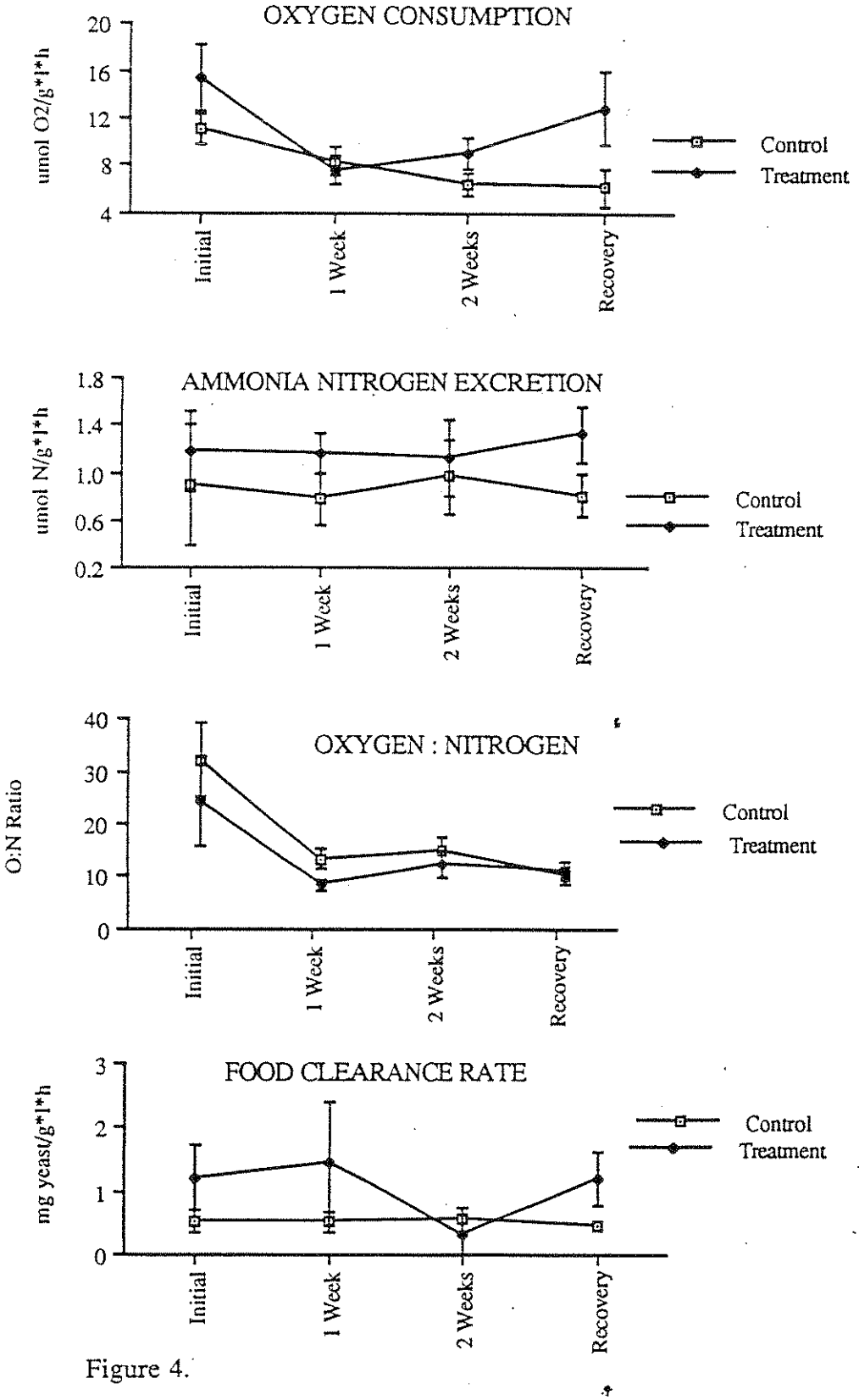

OXYGEN CONSUMPTION
umol O2/g*l*h
20
16
12
8
4
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
AMMONIA NITROGEN EXCRETION
umol N/g*l*h
1.8
1.4
1.0
0.6
0.2
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
OXYGEN : NITROGEN
O:N Ratio
40
30
20
10
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment
FOOD CLEARANCE RATE
mg yeast/g*l*h
3
2
1
0
Initial
1 Week
2 Weeks
Recovery
Control
Treatment

Figure 4.

Table 4. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates at 20° C for *Amblema plicata* before, during, and after exposure to 1000 ppm bentonite clay. A p value of .05 or less shows a difference between treatments when compared to initial measurements and n=6 per treatment.

Oxygen Consumption (μmol O_2/g*l*h)	Control	Treatment	p
Initial	11.07 (1.30)	15.38 (2.79)	
1 Week Exposure	8.35 (1.16)	7.59 (1.17)	.50
2 Weeks Exposure	6.43 (0.99)	8.98 (1.28)	.04
1 Week Recovery	6.18 (1.59)	12.77 (3.07)	.03
Ammonia Nitrogen Excretion (μmol N/g*l*h)	**Control**	**Treatment**	**p**
Initial	0.89 (0.51)	1.18 (0.34)	
1 Week Exposure	0.78 (0.22)	1.16 (0.17)	.21
2 Weeks Exposure	0.97 (0.31)	1.13 (0.32)	.51
1 Week Recovery	0.81 (0.18)	1.32 (0.24)	.08
Oxygen to Nitrogen Ratio	**Control**	**Treatment**	**p**
Initial	30.41 (7.06)	22.72 (8.52)	
1 Week Exposure	11.70 (1.85)	6.73 (0.99)	.06
2 Weeks Exposure	13.06 (2.49)	10.69 (2.42)	.54
1 Week Recovery	8.43 (1.59)	9.55 (1.42)	.61
Food Clearance Rate (mg yeast/g*l*h)	**Control**	**Treatment**	**p**
Initial	0.53 (0.17)	1.21 (0.51)	
1 Week Exposure	0.52 (0.16)	1.44 (0.97)	.12
2 Weeks Exposure	0.56 (0.19)	0.32 (0.32)	.38
1 Week Recovery	0.47 (0.06)	1.21 (0.43)	.01

Figure 5. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates for *Lampsilis radiata* maintained at the Animal Science gage station located at the upstream region of Little Pine Creek from 6/3/92 to 6/23/92.

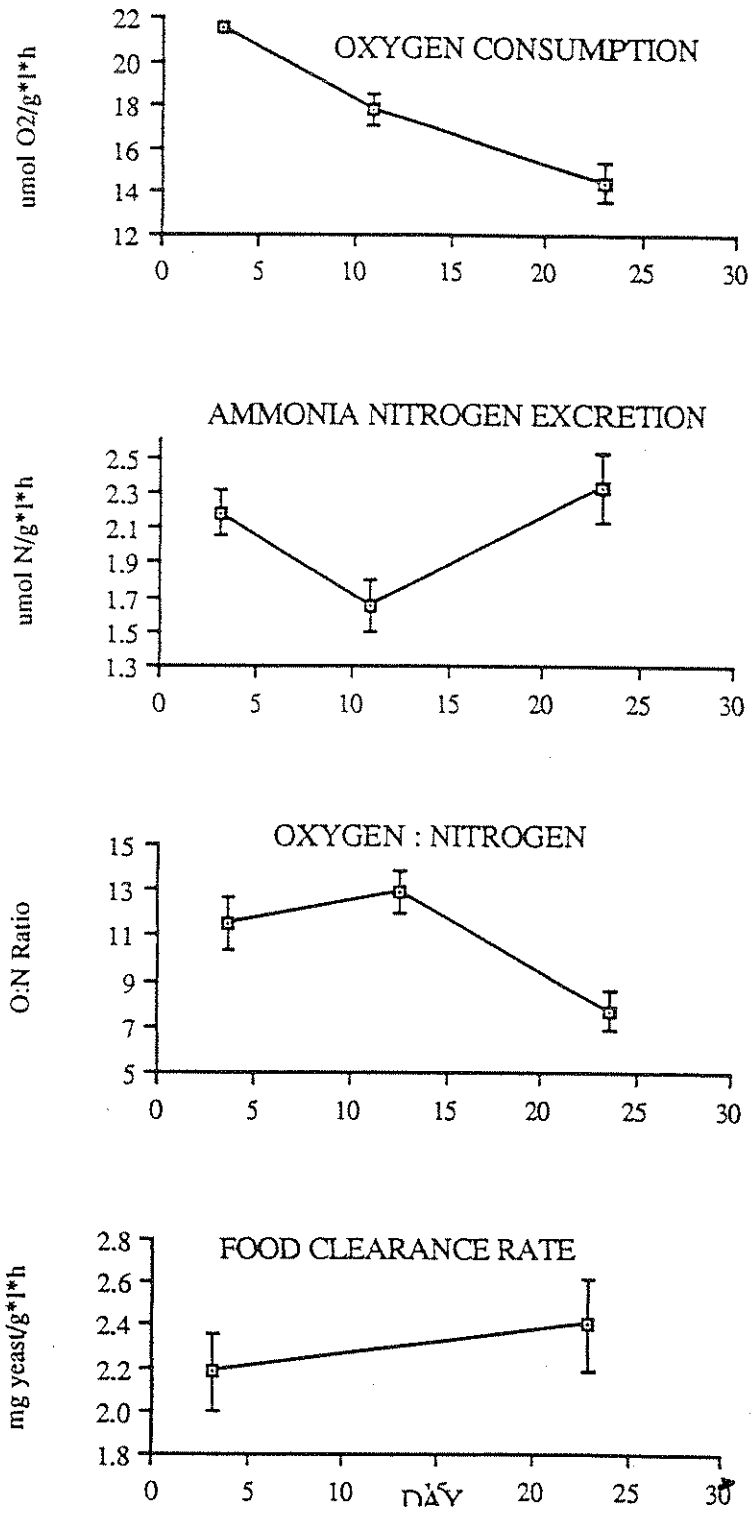

OXYGEN CONSUMPTION
umol O2/g*l*h
22
20
18
16
14
12
0
5
10
15
20
25
30
AMMONIA NITROGEN EXCRETION
umol N/g*l*h
2.5
2.3
2.1
1.9
1.7
1.5
1.3
0
5
10
15
20
25
30
OXYGEN : NITROGEN
O:N Ratio
15
13
11
9
7
5
0
5
10
15
20
25
30
FOOD CLEARANCE RATE
mg yeast/g*l*h
2.8
2.6
2.4
2.2
2.0
1.8
0
5
10
15
20
25
30

Figure 5.

Table 5. Comparison of mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates for *Lampsilis radiata* maintained at the gage station located at the upstream region of Little Pine Creek (Animal Science Farm) from 6/3/92 to 6/23/92. A p value of .05 or less shows a change in the parameter after one or two weeks when compared to the initial control parameter. Temperatures (^{o}C) are listed as the weekly means (SE) of hourly measures. n=29.

	Control (5/27)	(6/11)	p	(6/23)	p
Suspended Solids - Range Mean (mg/l)		35-214 77		36-540 131	
Temperature	14.22 (0.42)	15.72 (0.70)		14.56 (0.79)	
Oxygen Consumption (μmol O_2/g*l*h)	21.50 (0.19)	7.73 (0.73)	.01	14.45 (0.91)	.01
Ammonia Excretion (μmol N/g*l*h)	2.18 (0.13)	1.67 (0.15)	.01	2.33 (0.20)	.50
Oxygen to Nitrogen (O:N)	11.05 (1.13)	12.47 (0.96)	.30	7.26 (0.85)	.05
Food Clearance Rate (mg yeast/g*l*h)	2.18 (0.18)			2.40 (0.22)	.02

Oxygen consumption rates (Figure 6) decreased significantly after the first week and then returned to control levels. Ammonia nitrogen excretion rates (Figure 6) increased after one month in the stream water, and remained above control levels. Oxygen to nitrogen ratios (Figure 6) decreased, following the inverse pattern as the nitrogen excretion levels. Food clearance rates (Figure 6) increased in the mussels after the four week exposure period to the ambient water (Table 6).

Comparison of Mussel Metabolism at Upstream and Downstream Sites

Oxygen consumption rates in the mussels maintained at the Animal Science Farm gage (upstream) were lower than in those maintained at the Greenhill gage (downstream) ($p < .05$) (Figure 7). Food clearance rates were also lower in mussels at the Animal Science gage than those at Greenhill ($p<.05$) (Figure 7). Ammonia nitrogen excretion and oxygen to nitrogen ratios were not compared between the two groups because the inital measurements between the two groups were different ($p<.05$) (Table 7). The group maintained at the downstream region was collected from Crooked Lake in mid-May, 1992 while the group maintained at the upstream region was collected from the same location in late May, 1992. Typically, levels of suspended solids and nutrients are higher at the upstream site (Table A7). Although these trends were seen during this experiment, differences in these water quality characteristics between the two locations were not as great as was expected due to the small amount of rain during the experiment.

Metabolism of Field Collected Mussels and Laboratory Reared Mussels

The results from the three groups of *L. radiata* collected from Crooked Lake and measured independently show that all physiological parameters except the oxygen to nitrogen ratio decreased in mussels maintained under laboratory conditions for ten months (Table 8, Figure 8).

The two groups of freshly collected *Q. pustulosa* from the Tippecanoe River had similar oxygen consumption rates as the laboratory reared group (also collected from the Tippecanoe River). The group collected on 7/8/92 had greater ammonia nitrogen excretion rates than the laboratory-reared mussels but the group collected on 8/20/92 did not. Oxygen to nitrogen ratios were greater in the laboratory reared mussels than in the other groups. Food clearance rates differed among the three groups; the group collected on 7/8/92 had the largest rates and the group collected on 8/20/92 had the smallest rates (Table 9, Figure 9).

Figure 6. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates for *Lampsilis radiata* maintained at the gage station located at the downstream region of Little Pine Creek (Greenhill) from 5/25/92 to 6/25/92.

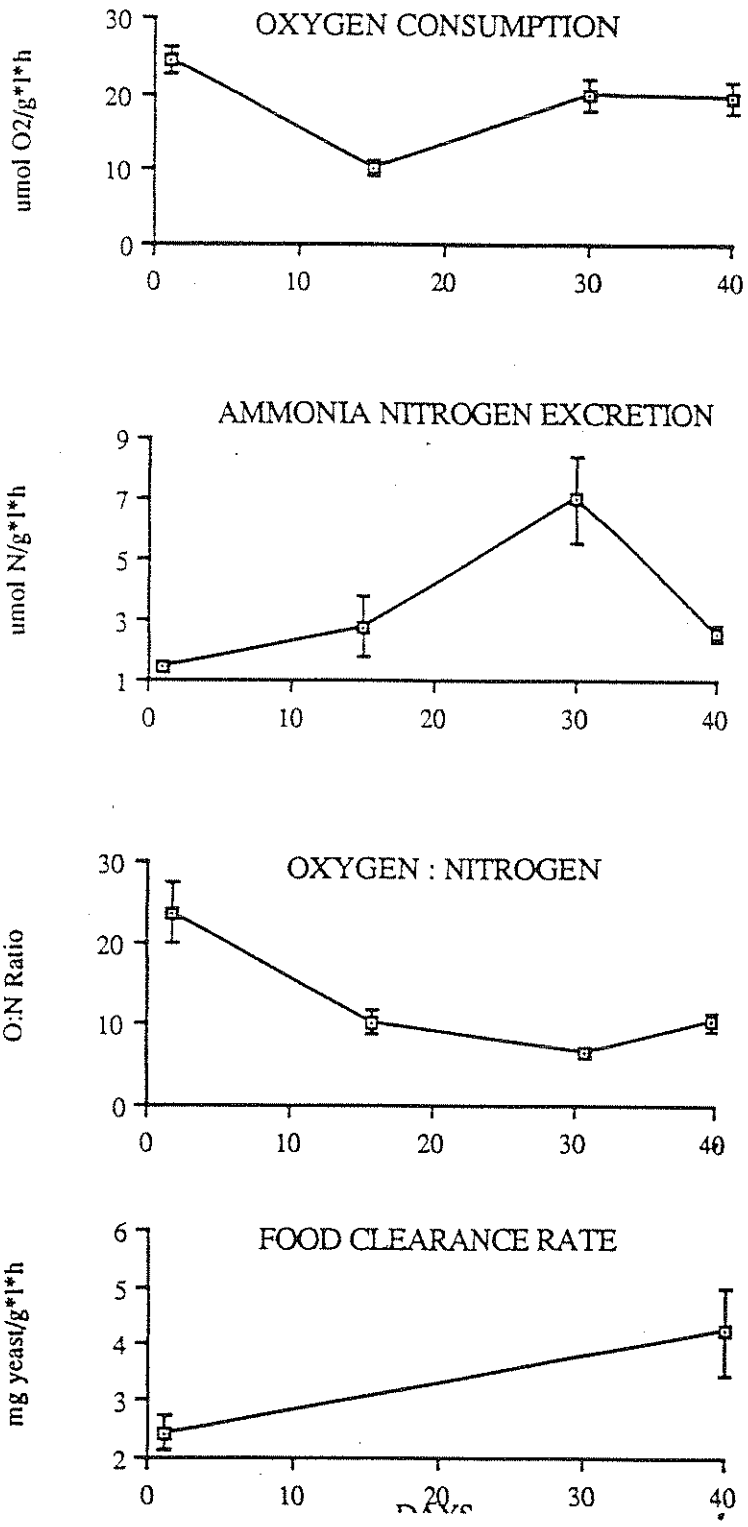
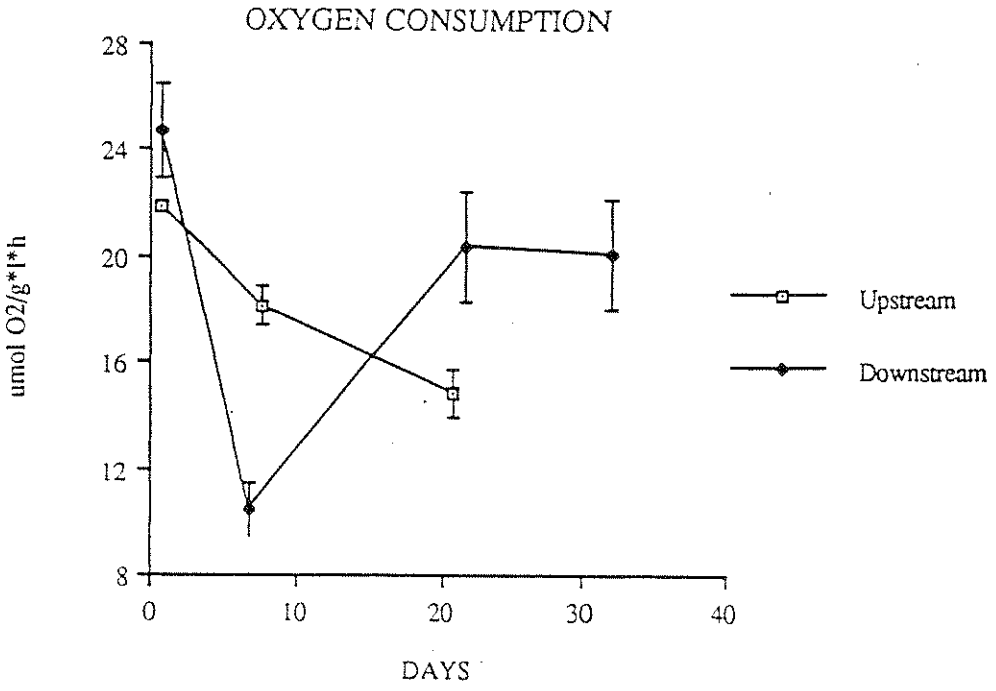

OXYGEN CONSUMPTION
umol O2/g*l*h
30
20
10
0
0
10
20
30
40
AMMONIA NITROGEN EXCRETION
umol N/g*l*h
9
7
5
3
1
0
10
20
30
40
OXYGEN : NITROGEN
O:N Ratio
30
20
10
0
0
10
20
30
40
FOOD CLEARANCE RATE
mg yeast/g*l*h
6
5
4
3
2
0
10
20
30
40

Figure 6.

Table 6. Mean (SE) oxygen consumption (VO_2 - µmol O_2/g*l*h), ammonia nitrogen excretion (NE - µmol N/g*l*h), oxygen to nitrogen ratios (O:N), and food clearance rates (FCR - mg yeast/g*l*h) for *Lampsilis radiata* maintained at the Greenhill gage station located at the downstream region of Little Pine Creek from 5/25/92 to 6/25/92. A p value of .05 or less shows a change in the parameter when compared to the initial control parameter. Temperatures (°C) are listed as weekly means (SE) of hourly measurements. n=21.

Date	5/15	5/31	6/15	6/25
Suspended Solids - Range		9 - 14	3 - 20	
mean (ppm)		12	12	2
Temperature (0.50)	14.14 (0.82)	19.93 (0.60)	20.39 (0.43)	24.56
VO_2	24.33 (1.80)	10.04 (1.07)	20.01 (2.08)	19.69 (2.11)
p		.01	.14	.06
NE	1.45 (0.18)	2.77 (1.03)	6.97 (1.43)	2.57 (0.25)
p		.30	.01	.01
O:N	22.23 (3.73)	8.85 (1.43)	5.24 (0.77)	9.05 (1.09)
p		.01	.01	.01
FCR	2.43 (0.31)			4.21 (0.75)
p				.03

Figure 7. Mean (SE) oxygen consumption and food clearance rates for *Lampsilis radiata* maintained at the Animal Science gage station (upstream) and at the Greenhill gage station (downstream).


OXYGEN CONSUMPTION
umol O2/g*l*h
28
24
20
16
12
8
0
10
20
30
40
DAYS
Upstream
Downstream

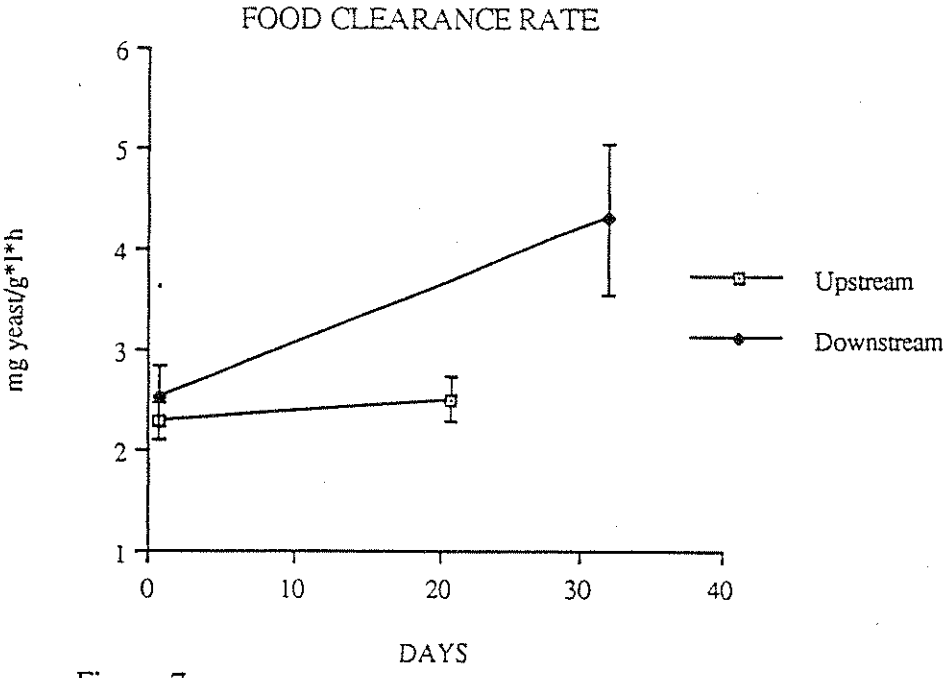

FOOD CLEARANCE RATE
mg yeast/g*l*h
6
5
4
3
2
1
0
10
20
30
40
DAYS
Upstream
Downstream

Figure 7.

Table 7. Mean (SE) oxygen consumption, ammonia nitrogen excretion, oxygen to nitrogen ratios, and food clearance rates for *Lampsilis radiata* maintained at the Animal Science Farm gage station (upstream) and at the gage station located at Greenhill (downstream). A p value of .05 or less shows a difference between the two groups. Water quality measurements (mg/l) are means of weekly grab samples taken during the experiment. n=50.

Oxygen Consumption (μmol O_2/g*l*h)

DATE	DAIRY FARM	DATE	GREENHILL	p value
5/27	21.50 (0.19)	5/15	24.33 (1.80)	.22
6/11	17.73 (0.73)	6/15	20.01 (2.08)	.25
6/23	14.45 (0.91)	6/25	19.69 (2.11)	.02

Ammonia Nitrogen Excretion (μmol N/g*l*h)

DATE	DAIRY FARM	DATE	GREENHILL	p value
5/27	2.18 (0.13)	5/15	1.45 (0.18)	.01
6/11	1.67 (0.15)	6/15	6.97 (1.43)	.01
6/23	2.33 (0.20)	6/25	2.57 (0.25)	.40

Oxygen to Nitrogen Ratio

DATE	DAIRY FARM	DATE	GREENHILL	p value
5/27	11.05 (1.13)	5/15	22.23 (3.73)	.01
6/11	12.47 (0.96)	6/15	5.24 (0.77)	.01
6/23	7.26 (0.85)	6/25	9.05 (1.09)	.31

Food Clearance Rate (mg/g*l*h)

DATE	DAIRY FARM	DATE	GREENHILL	p value
5/27	2.18 (0.18)	5/15	2.43 (0.31)	.46
6/23	2.40 (0.22)	6/25	4.21 (0.75)	.01

	DAIRY FARM	GREENHILL
Mean Suspended Solids	104	9
Nitrate	20.0	6.0
Nitrite	0.65	0.21
Phosphate	0.01	0.06

Figure 8. Mean (SE) oxygen consumption rate, ammonia nitrogen excretion rate, oxygen to nitrogen ratio, and food clearance rate for "wild" *Lampsilis radiata* (collected from Crooked Lake on 5/14/92 and 5/27/92) and for "lab" reared *Lampsilis radiata* (collected from Crooked Lake on 5/16/91) that were maintained under laboratory conditions for ten months. All measurements were taken at 20°C in unchlorinated well water.

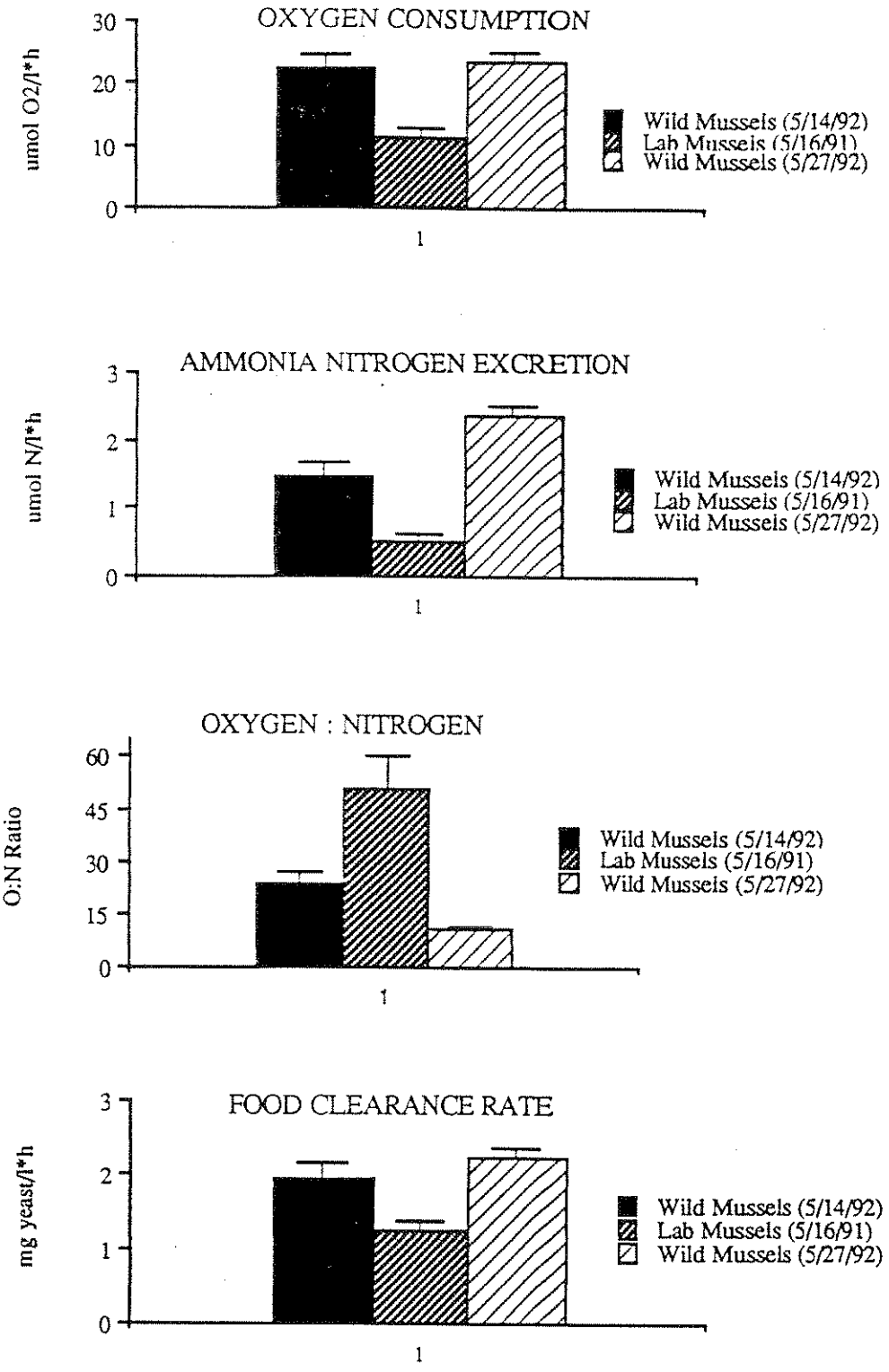

OXYGEN CONSUMPTION
umol O2/l*h
30
20
10
0
1
Wild Mussels (5/14/92)
Lab Mussels (5/16/91)
Wild Mussels (5/27/92)
AMMONIA NITROGEN EXCRETION
umol N/l*h
3
2
1
0
1
Wild Mussels (5/14/92)
Lab Mussels (5/16/91)
Wild Mussels (5/27/92)
OXYGEN : NITROGEN
O:N Ratio
60
45
30
15
0
1
Wild Mussels (5/14/92)
Lab Mussels (5/16/91)
Wild Mussels (5/27/92)
FOOD CLEARANCE RATE
mg yeast/l*h
3
2
1
0
1
Wild Mussels (5/14/92)
Lab Mussels (5/16/91)
Wild Mussels (5/27/92)

Figure 8.

Table 8. Comparison of mean (SE) oxygen consumption rate (VO_2), ammonia nitrogen excretion rates (ANE), oxygen to nitrogen ratios (O:N), and food clearance rates (FCR) between individuals of *Lampsilis radiata* maintained under laboratory conditions for ten months and those recently collected. Lab-reared mussels (n=21) were collected from Crooked Lake in May 1991 and "wild" mussels were collected in mid (n=21) and late (n=27) May, 1992 from Crooked Lake. All measurements were taken at 20°C in unchlorinated well water. A one-way ANOVA and Student-Neman-Keuls (SNK) test compared lab mussels with wild mussels caught on 5/14/92 (p^1) and lab mussels with wild mussels caught on 5/27/92 (p^2). A p value of .05 or less shows a difference between the groups.

Wild Mussels (5/14/92)	p^1	Lab Mussels (5/16/91)	p^2	Wild Mussels (5/27/92)
VO_2 (µmol O_2/l*h)				
22.38 (2.40)	.01	11.47 (1.29)	.01	23.60 (1.45)
ANE (µmol N/l*h)				
1.48 (0.20)	.01	0.51 (0.13)	.01	2.38 (0.13)
O:N				
23.51 (3.74)	.01	50.57 (9.70)	.01	10.66 (1.18)
FCR (mg yeast/l*h)				
1.94 (0.24)	.01	1.24 (0.13)	.01	2.24 (0.11)

Figure 9. Mean (SE) oxygen consumption rate, ammonia nitrogen excretion rate, oxygen to nitrogen ratio, and food clearance rate for "wild" *Quadrula pustulosa* (collected from the Tippecanoe River on 7/13/92 and 8/23/92) and for "lab" reared *Quadrula pustulosa* (collected from the Tippecanoe River on 9/17/91) and maintained under laboratory conditions for ten months. All measurements were taken at 20°C in unchlorinated well water.

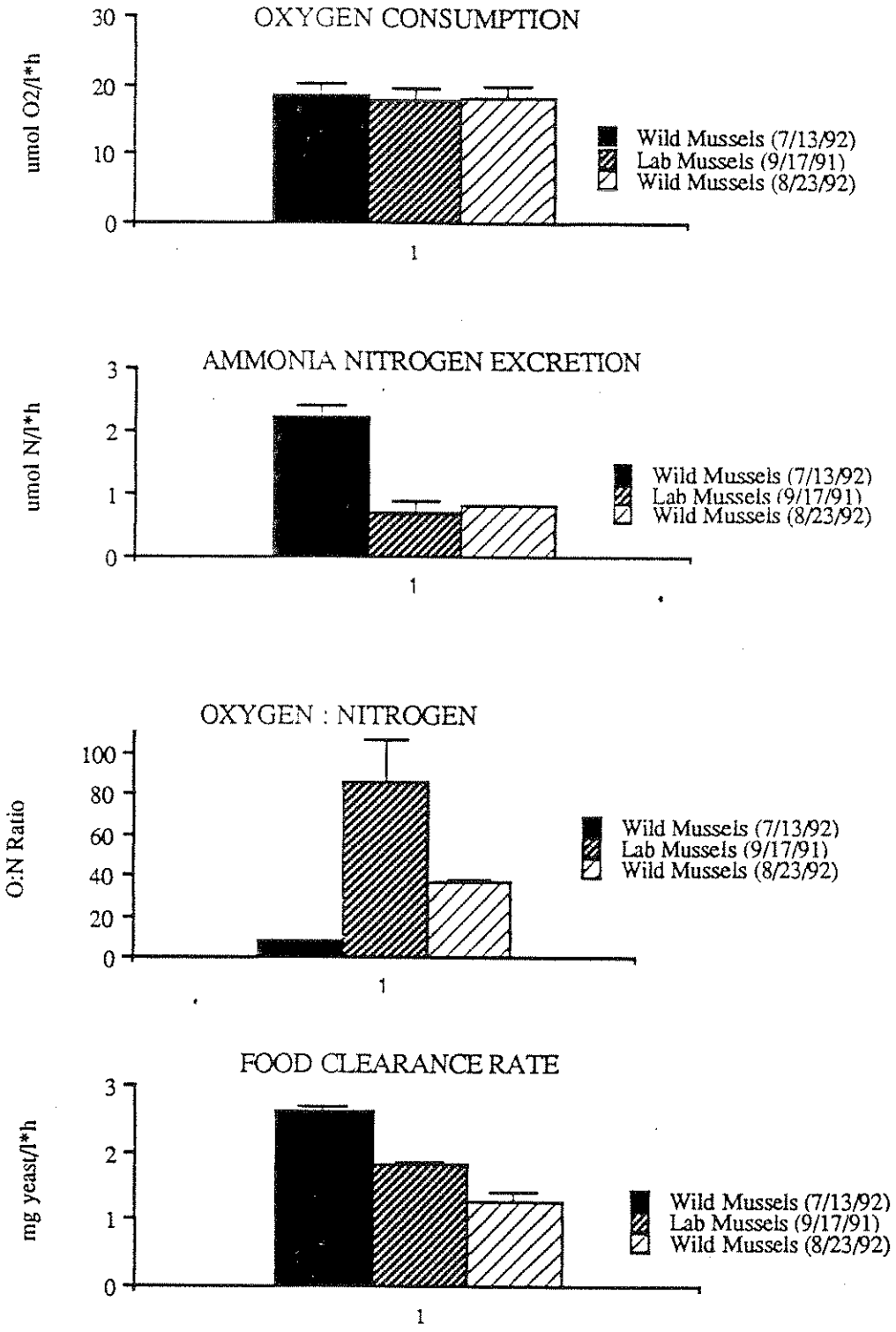

OXYGEN CONSUMPTION
umol O2/l*h
30
20
10
0
1
Wild Mussels (7/13/92)
Lab Mussels (9/17/91)
Wild Mussels (8/23/92)
AMMONIA NITROGEN EXCRETION
umol N/l*h
3
2
1
0
1
Wild Mussels (7/13/92)
Lab Mussels (9/17/91)
Wild Mussels (8/23/92)
OXYGEN : NITROGEN
O:N Ratio
100
80
60
40
20
0
1
Wild Mussels (7/13/92)
Lab Mussels (9/17/91)
Wild Mussels (8/23/92)
FOOD CLEARANCE RATE
mg yeast/l*h
3
2
1
0
1
Wild Mussels (7/13/92)
Lab Mussels (9/17/91)
Wild Mussels (8/23/92)

Figure 9.

Table 9. Comparison of mean (SE) oxygen consumption rates (VO_2), ammonia nitrogen excretion rates (ANE), oxygen to nitrogen ratios (O:N), and food clearance rates (FCR) between individuals of *Quadrula pustulosa* maintained under laboratory conditions for ten months and those recently collected. Lab-reared mussels (n=22) were collected from the Tippecanoe River in September, 1991 and "wild" mussels were collected in mid July (n=30) and in late August (n=30), 1992. All measurements were taken at 20°C in unchlorinated well water. A one-way ANOVA and Student-Newman-Keuls (SNK) test compared lab mussels with wild mussels caught on 7/13/92 (p^1) and lab mussels with wild mussels caught on 8/23/92 (p^2). A p value of .05 or less shows a difference between the groups and NS (not significant) shows no difference between the groups.

	Wild Mussels (7/13/92)	p^1	Lab Mussels (9/17/91)	p^2	Wild Mussels (8/23/92)
VO_2 (µmol O_2/l*h)	18.53 (1.86)	NS	17.91 (1.74)	NS	18.07 (1.97)
ANE (µmol N/l*h)	2.24 (0.18)	.01	0.72 (0.16)	NS	0.80 (0.03)
O:N	7.77 (0.51)	.01	86.09 (20.16)	.01	36.19 (1.99)
FCR (mg yeast/l*h)	2.61 (0.09)	.01	1.80 (0.05)	.01	1.25 (0.14)

DISCUSSION

In the laboratory experiments, physiological parameters were analyzed for changes over time when compared to initial measurements and between treatments (control and suspended solid exposure). Mussels in the suspended solid treatment rarely had significantly different parameters than those in the control conditions. Changes in the physiological parameters did occur over time, however. The intial measurements represent the most normal, healthy metabolic measurements in each of the species. When maintained under laboratory conditions (in experimental aquaria or in "living streams"), health of all of the species declined and changes in metabolic parameters were observed. Oxygen consumption rates decreased under laboratory conditions when compared to intial rates, except for the group of *Q. pustulosa* that was maintained under laboratory conditions for ten months. Ammonia nitrogen excretion rates were significantly lower in the groups of *Q. pustulosa* and *L. radiata* that were maintained under laboratory conditions than the initial rates in recently collected individuals. Ammonia nitrogen excretion rates decreased over time in most of the three week experiments as well. Oxygen to nitrogen ratios generally increased over time during the three week experiments and were significantly greater and above 30 in the groups of *Q. pustulosa* and *L. radiata* that were maintained for ten months under laboratory conditions. Food clearance rates were significantly lower in the mussels maintained under laboratory conditions for extended periods and tended to decrease over time during the three week experiments. The length of time mussels were maintained under laboratory conditions, rather than suspended solid exposure, induced the metabolic changes that others have measured in response to stress in unionids (Aldridge et al. 1987).

The physiological parameters measured in *Q. pustulosa* and *A. plicata* over the three week experimentation period of suspended solids exposure (500 and 1000 ppm) and recovery were more variable over time than expected and surprising in some cases. Physiological parameters of mussels in the treatment groups were not usually different from those of the control groups. One exception was the decreased food clearance rate in *A. plicata* in response to 500 ppm bentonite clay. Food clearance rates were less than controls after one and two weeks and returned to initial control levels after the one week recovery period (Figure 3). Because this response was not seen when *A. plicata* was exposed to 1000 ppm bentonite clay, a reduction in

food clearance rate does not appear to be a reliable or reproducible response to suspended solids stress. Similarly, the food clearance rate decreased in *Q. pustulosa* after one week of exposure to 1000 ppm bentonite clay; although this parameter continued to decrease after the second week of exposure, it was not different from control levels during the second week. As with *A. plicata*, food clearance rates returned to initial control levels after the one week recovery period (Figure 2). Naimo et al. (1992) found that oxygen consumption rates and food clearance rates were more predictable and reliable than ammonia nitrogen excretion rates and oxygen to nitrogen ratios as indicators of cadmium stress in *L. ventricosa*. Although food clearance rate was the most predictable parameter in this study, it did not consistently indicate a stress response in *Q. pustulosa* and *A. plicata* after exposure to 500 and 1000 ppm suspended solids. Oxygen consumption rates and food clearance rates may be more reliable measures of stress in *L. radiata* (Figure 7).

Results from the experiment in which *A. plicata* was exposed to 1000 ppm bentonite clay were unexpected. Although initial measurements between control and treatment groups did not differ significantly, the control parameters were consistently lower than the treatment parameters throughout the experiment. After one week of exposure, the mean oxygen consumption rates of the treatment group decreased from initial levels, but were not different from the relatively lower control group levels. After the second week of exposure and the one week recovery, the control mean oxygen consumption rates were lower than the rates in treatment group. Similarly, the food clearance rates in the control group appeared to be lower than the treatment levels throughout the entire experiment, and were significantly lower after the one week recovery period. Ammonia nitrogen excretion rates followed a similar trend (levels appeared to be lower in the control group) as did the oxygen to nitrogen ratios (levels appeared to be higher in the control group) throughout this experiment (Figure 4). A perplexing result from the experiment where *Q. pustulosa* was exposed to 500 ppm bentonite clay was that ammonia nitrogen excretion rates were lower in the control group than in the treatment group, not initially, but after the first, second, and third weeks.

A trend consistent throughout the four laboratory exposure experiments is that although treatment group parameters often changed after exposure to suspended solids as predicted (with declining oxygen consumption rates and ammonia nitrogen excretion rates, and increasing oxygen to nitrogen ratios) the control group parameters followed a similar trend, so that differences over time of exposure were not seen when compared to controls. These results indicate that mussel health declines consistently over time when maintained in culture conditions (Figure 5). Naimo et al. (1992) also found a decline in unionid health under laboratory conditions. Although only freshly collected mussels were used in the experiments

in an attempt to avoid this problem, mussel health decline appears to be immediate. Perhaps unionids require more natural prey items or flow conditions to maintain normal metabolic patterns.

Decline in health is evident from the comparison of measurements taken on laboratory cultured individuals to initial measurements taken on two groups of freshly collected individuals of *L. radiata* . Oxygen consumption rates, ammonia nitrogen excretion rates, and food clearance rates were significantly lower in the laboratory cultured individuals than in the two groups of freshly collected mussels. Oxygen to nitrogen ratios were greater in the cultured mussels than in the freshly collected mussels (Table 8, Figure 8) suggesting that the laboratory held mussels had swithed from protein based catabolism (used under healthy environmental conditions in unionids) and were using alternate stored carbohydrate or lipid reserves for energy. Although the cultured mussels were fed regularly and maintained under what we considered good water quality conditions, their health as measured by these parameters was not as good as the health of the freshly collected individuals.

Results from similar analyses of laboratory cultured and freshly collected *Q. pustulosa* are not as straight forward. Oxygen consumption rates were not different between the two groups of freshly collected mussels and the laboratory cultured group (Figure 9a). When comparing only the wild mussels collected on 7/13/92 with the laboratory cultured mussels, all of the other parameters follow the trend seen with *L. radiata*, supporting the conclusion that health declines in captivity. Ammonia nitrogen excretion rates were greater in the wild mussels ($p<.01$), oxygen to nitrogen ratios were less in the wild mussels ($p<.01$) and food clearance rates were greater in the wild mussels ($p<.01$) (Figure 9). However, the group of mussels collected on 8/23/92 had relatively low ammonia nitrogen excretion rates which were not different from that of the laboratory cultured mussels, and their food clearance rates were actually lower than those of the laboratory cultured mussels ($p<.01$). Oxygen to nitrogen ratios, however, were lower in the wild mussels than in the laboratory cultured mussels ($p<.01$) (Table 9). These results suggest that the group of mussels taken from the Tippecanoe River in mid July were healthier than the group taken from the same location in late August. The reproductive condition of these individuals should not have been different, since this species remains gravid from June to August (Cummings and Berlocher 1990). Possibly, water conditions changed over this time causing the mussels collected in August to be more stressed than the group collected in July. An initial concern with the experimental design was that the mussels would recover quickly when they were removed from their treatment and placed in water free of suspended solids for the measurement of the various parameters. It seems plausible that mussels taken from the suspended solids treatment could recover immediately and filter water, removing oxygen and

food, faster than control levels. The only experiment in this study that may support this hypothesis is the experiment where *A. plicata* was exposed to 1000 ppm bentonite clay (Table 4). If this artifact does exist, it should not present a problem when measuring ammonia nitrogen excretion, since this parameter is an indirect measure of how much the mussel is feeding over time. Theoretically, ammonia nitrogen excretion should be a good parameter, however, it has been shown to be a highly variable, and often unpredictable parameter in this study and others (Naimo et al. 1992).

The ambient exposure experiments, where individuals of *L. radiata* were placed at a first order region of Little Pine Creek (Animal Science Farm) and at a third order region of Little Pine Creek (Greenhill), were dissappointing because the suspended solids concentrations never reached continuously high levels due to the absence of rain during this time (May and June 1992). Results are useful, however, in looking at mussel health over time under these ambient conditions. Water quality and temperature at the two locations were different; nitrate concentrations were as much as twenty times higher at the Animal Science Farm than at Greenhill. Oxygen consumption rates decreased after one and two weeks at the Animal Science Farm ($p<.05$) but the other parameters did not change over time or remained within healthy ranges (Figure 5). Similarly, parameters increased and decreased over time in the mussels maintained at Greenhill, but remained within healthy ranges overall (Figure 6).

Oxygen consumption rates and food clearance rates were greater in mussels maintained at Greenhill than in those at the Animal Science Farm, possibly indicating that the mussels at Greenhill were healthier than those at the Animal Science Farm (Figure 7). However, changes in ammonia nitrogen excretion rates and oxygen to nitrogen ratios in the group at the Animal Science Farm did not indicate that the mussels were stressed. The greater oxygen consumption and food clearance rates in the mussels at the downstream site may have resulted from the higher average daily water temperatures at that site than at the upstream site; average weekly water temperatures at the downstream site were 19.8 ^{o}C while the average temperatures at the upstream site were 14.8 ^{o}C (Table 7). Metabolic rate likely increases with increasing temperature in unionids. Lewis (1984) showed that oxygen consumption rates increased in two unionid species as water temperature increased.

Naimo et al. (1992) found these parameters to be fairly reliable indicators of cadmium stress in *L. ventricosa*. Possibly species in the genus *Lampsilis* are more prone to decrease oxygen consumption rates and food clearance rates when exposed to environmental stress than are *Q. pustulosa* or *A. plicata*. (There were not convincing decreases in oxygen consumption or food clearance rates in *Q. pustulosa* or *A. plicata* after exposures to suspended solids.) They

proposed that the reduced oxygen consumption rates resulted from an increased mucus coating over the gill which decreased oxygen extraction efficiency. In response to the decreased oxygen consumption rates, the mussels reduced their metabolic rate and decreased food intake. It is also possible that the particle sorting capacity of the gills was reduced due to the greater mucus coating in response to the cadium exposure. It is not clear why species of *Lampsilis* would respond differently to suspended solids or cadmium stress than other unionid species; although species in the genus *Lampsilis* have a larger mantle flap used for display and a larger foot muscle than other unionid species, the filtering mechanisms in unionids are the same.

None of the species maintained under control or treatment conditions had noticeable gill mucus secretions, but the mussels from the treatment groups did "clear" suspended solids from their gills when placed in clean water for measurement of the physiological parameters. It seems highly plausible that suspended solids taken into the gills reduces oxygen extraction efficiency and particle separating capabilities simply by reducing surface area of the gills. If this phenomenon occurred, it was not significant enough to reduce oxygen consumption or food clearance rates consistently or to secondarily reduce ammonia nitrogen excretion rates (by reducing food consumption). Aldridge et al. (1987) found reductions in all of these parameters after exposure to 600 to 750 ppm diatomaceous earth and turbulence in *Q. pustulosa* and two other unionid species. Possibly the diatomaceous earth, which is more abrasive than bentonite clay, damaged the gill lamellae, causing the reduction in all of the parameters and the consequential increase in oxygen to nitrogen ratios. Aldridge et al. (1987) did not discuss a feeding regimen for the mussels during the experiment. Starvation increases oxygen to nitrogen ratios in gastropods (Russell-Hunter 1983) and may have contributed to the effects of the diatomaceous earth and turbulence to increase oxygen to nitrogen ratios in the mussels in that study.

Future Research Needs

Changes in oxygen consumption rates, ammonia nitrogen excretion rates, oxygen to nitrogen ratios, and food clearance rates in unionids in response to stress are not yet understood well enough for use in management of unionid populations. To reach that point, a range of physiological parameters under normal, healthy conditions must be defined for each species under investigation. This study reported healthy metabolic parameters measured after collection in *L. radiata*, *Q. pustulosa*, and *A. plicata*. But even these initial measurements were somewhat variable. Seasonal variation may cause large shifts in these parameters. Ammonia nitrogen excretion rates and food clearance rates differed in two groups of *Q. pustulosa*

collected from the same region of the Tippecanoe River over a one month period. To better understand the effects of seasonal variation on these physiological parameters, measurements could be taken monthly on marked individuals maintained in their normal habitat for at least a year.

In addition, the results from this study did not determine that the mussels converted to an anaerobic mode of metabolism when maintained in water with suspended solids. A simple way to answer this question would involve modifying the laboratory experiments so that oxygen consumption measurements be taken under the treatment conditions; mussels maintained in suspended solids would be placed in a respirometer with suspended solids and mussels maintained under control conditions would be placed in a respirometer with clear water. If suspended solids do not induce anaerobiosis in mussels, then mussels in water with suspended solids should remove oxygen as efficiently as mussels in clear water.

Conversely, if the mussels from the treatment do not filter oxygen while maintained under the treatment conditions, then the mussels could have been "recovering" from the treatment conditions when the physiological measurements were taken in this study. In this case, the extent of anaerobic metabolism that had occurred over time in the mussels could be determined by measuring tissue lactic acid content. To determine which tissues are degraded preferentially under suspended solids stress, tissue protein, carbohydrate, and lipid contents could be analyzed in subsamples taken initially and after different lengths of exposure.

If such studies prove these metabolic parameters too variable for use as an index of stress in unionids, more sensitive indices should be developed. RNA-DNA ratios are used to measure growth in animals because the amount of cellular DNA is constant while the amount of RNA varries depending on how rapidly the cells divide and grow. RNA-DNA ratios have been used as indicators of feeding, reproduction, and energy storage in wild populations of fish (Bulow et al. 1981) as well as indicators of growth in fish exposed to low levels of an insecticide (Wilder and Stanley 1983). Measuring mussel growth with standard techniques of weight, length and width changes over time is highly imprecise and is not used because of the slow growth rate of mussels. RNA-DNA ratios may be useful in measuring growth and possibly general health in mussels. Adult mussels may grow too slowly for this technique to be a useful measure of health, but if tissue samples for analysis could be taken from individuals without sacrificing the individuals, the technique should be tried.

Another important research need is the determination of habitat requirments of unionids. In many cases, it will be essential that unionids be studied and maintained under laboratory

conditions. As my results and the results of Naimo et al. (1992) showed, unionid health declines rapidly under the currently employed culture conditions. If unionid requirements were more precisely defined, culture condions could be improved to better meet their requirements. Because progress has been made in culturing glochidea without using fish hosts (Keller and Zam 1990; Isom and Hudson 1982), this information could be especially useful in maintaining broodstocks under culture conditions.

Likely requirements of unionids that are not being met under laboratory conditions include substrate characteristics, current velocity, and nutritional quality or accesibility of food. Different species have different niches (Cummings and Berlocher 1990) and therefore, some species may adapt better to laboratory culture conditions than other species. Experiments to determine essential environmental requirements of different species under laboratory conditions are needed before further laboratory studies designed to look at other problems can be successfully completed.

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APPENDIX

Figure A1. Regression of wet weight (y) in grams and length of empty shells (x) in millimeters ($y = 59.78 + 0.55x$; $r^2 = 0.796$) in *Lampsilis radiata*.

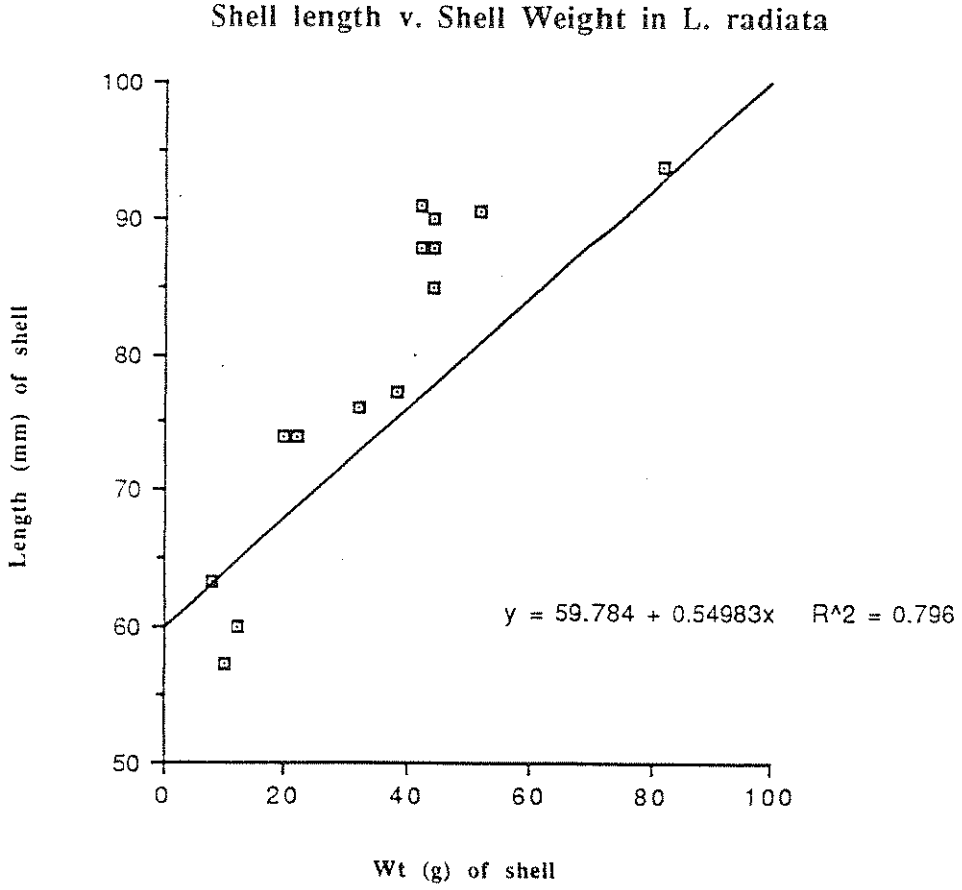
Shell length v. Shell Weight in L. radiata
Length (mm) of shell
Wt (g) of shell
y = 59.784 + 0.54983x R^2 = 0.796
100
90
80
70
60
50
0
20
40
60
80
100

Figure A1.

Table A1. Wet live weight (shell and flesh) and dry weight (flesh) and shell weight for *Lampsilis radiata* collected on 5/14/92 from Crooked Lake. Wet weights were taken a few days after collection and the flesh weight of each individual was obtained approximately three weeks later by drying to constant weight at 75°C for two days. All measurements are in grams.

Individual	Wet Live Weight	Dry Flesh Weight	Shell Weight
1	62	1.9	31.7
2	50	0.8	23.5
3	42	1.3	18.1
4	56	1.4	27.3
5	36	1.1	16.5
6	30	0.7	11.3
7	30	0.8	14.0
8	40	1.2	17.5
9	32	0.9	14.2
10	40	1.2	14.8
11	58	1.6	26.9
12	30	0.7	12.5
13	80	2.0	40.3
15	30	0.8	12.3
16	28	0.8	10.5
17	30	0.7	11.3
18	24	0.6	10.4
19	12	0.3	6.4
20	8	0.3	3.4
21	10	0.4	5.4
22	6	0.3	3.4
23	10	0.3	5.6

Table A2. Wet live weight (shell and flesh) and dry weight (flesh) and shell weight for *Lampsilis radiata* collected on 5/27/92 from Crooked Lake. Wet weights were taken a few days after collection and the flesh weight of each individual was obtained approximately three weeks later by drying to constant weight at 75°C for two days. All measurements are in grams.

Individual	Wet Live Weight	Dry Flesh Weight	Shell Weight
1	32	1.1	13.4
2	40	1.3	16.2
3	32	1.0	14.7
4	44	1.7	18.4
5	40	0.8	16.4
6	24	0.7	10.1
7	28	0.8	12.4
8	42	1.7	18.8
9	34	0.8	13.7
10	50	1.5	21.3
11	42	1.0	17.3
15	38	1.0	13.5
16	46	1.1	21.8
17	26	0.9	10.3
18	54	1.3	21.0
20	54	1.9	23.6
23	28	0.8	10.9
24	62	1.5	26.1
25	50	1.2	11.6
26	58	1.6	19.9
28	22	0.5	4.8
29	46	1.1	22.4
30	32	1.0	15.2
31	32	1.0	15.7
33	32	1.0	12.2
34	50	1.4	19.5
35	52	1.7	21.9
36	48	1.5	21.0
37	26	0.7	11.1

Table A3. Wet live weight (flesh and shell) and dry flesh weight (flesh) for *Quadrula pustulosa* collected on 7/8/92 from the Tippecanoe River. Wet weights were taken a few days after collection and the flesh weight of each individual was obtained approximately three weeks later by drying to constant weight at 75°C for two days. All weights are in grams.

Individual	Wet Live Weight	Dry Flesh Weight
1	78	1.6
2	80	1.6
3	56	1.8
4	148	4.3
5	64	1.2
6	126	3.1
7	62	1.7
8	60	1.4
9	86	1.9
10	56	1.4
11	68	1.8
12	86	2.0
13	66	1.6
14	88	2.5
15	142	4.5
16	98	2.3
17	28	0.6
18	24	.06
19	28	0.8
20	8	0.2
21	10	0.4
22	134	2.5
23	168	5.0
24	82	2.1
25	132	4.4
26	24	0.6
27	162	6.9
28	60	1.4
29	56	1.8
30	16	0.4

Table A4. Wet live weights (flesh and shell) and dry flesh weights for *Quadrula pustulosa* collected on 8/20/92 from the Tippecanoe River. Wet weights were taken a few days after collection and dry weights were taken approximately 3 weeks later by drying the flesh to constant weight. All measurements are in grams.

Individual	Live Wet Weight	Dry Flesh Weight
1	50	1.3
2	60	1.4
3	100	2.1
4	66	1.2
5	114	2.5
7	58	0.9
8	32	0.4
9	142	2.0
10	10	0.1
11	48	1.1
12	152	3.4
13	108	2.4
14	106	2.8
15	80	1.3
16	70	1.8
17	124	2.9
18	96	2.1
19	86	2.0
20	152	3.6
21	78	1.8
22	86	1.9
23	168	4.4
24	104	2.7
25	62	1.5
26	64	1.2
27	94	1.9
28	128	2.9
29	74	1.5
30	58	1.0
31	118	2.1

Table A5. Wet live weights (flesh and shell), dry flesh weights, and shell weights for *Amblema plicata* collected from the Tippecanoe River on 7/8/92. Wet weights were taken a few days after collection and the flesh weight of each individual was obtained approximately three weeks later by drying to a constant weight at 75°C for two days. All measurements are in grams.

Individual	Wet Live Weight	Dry Flesh Weight
1	346	6.7
2	36	0.7
3	48	0.9
4	48	0.9
5	26	0.7
6	20	0.5
7	40	1.2
8	54	1.1
9	114	2.7
10	196	4.2
11	426	10.5
12	440	8.9

Table A6. Wet live weights (flesh and shell) and dry flesh weights for *Amblema plicata* collected from the Tippecanoe River on 8/20/92. Wet weights were taken a few days after collection and the flesh weight of each individual was obtained approximately three weeks later by drying to a constant weight at 75°C for two days. All measurements are in grams.

Individual	Wet Live Weight	Dry Flesh Weight
1	552	9.1
2	300	6.1
3	292	6.6
4	258	5.7
5	222	4.1
6	176	3.1
7	138	2.6
8	162	2.2
9	114	2.3
10	116	2.1
11	64	1.2
12	44	0.6

Table A7. Repeated, paired, individual oxygen consumption and ammonia nitrogen excretion rates for laboratory cultured individuals of *Quadrula pustulosa* and *Lampsilis radiata* maintained under control conditions.

Oxygen Consumption Rates (µmol O_2/l*h)

Quadrula pustulosa		*Lampsilis radiata*	
3/16/92	3/24/92	3/16/92	3/24/92
17.74	14.78	5.91	4.45
17.74	11.83	8.87	2.97
20.69	20.69	10.35	8.75
25.13	17.74	10.35	11.75
23.65	17.74	17.74	11.83
22.17	23.50	20.69	20.69
7.39	0.0	7.39	0.0
10.35	11.75	23.65	17.74

Ammonia Nitrogen Excretion Rates (µmol N/l*h)

Quadrula pustulosa		*Lampsilis radiata*	
3/8/92	3/16/92	3/6/92	3/13/92
1.15	0.0	0.71	0.56
0.49	0.78	0.71	0.56
1.44	1.0	0.56	0.56
0.78	0.41	1.29	0.12
0.19	0.85	1.07	1.44
0.41	0.41	2.91	0.0
1.15	1.44	5.71	0.85
0.85	0.26	0.0	0.34
1.44	0.26	1.88	0.0
0.19	0.26	0.85	1.29
1.15	0.56	0.56	0.56
1.59	0.41	0.0	0.41
0.34	0.34	0.26	0.19
0.26	2.47	0.71	0.71
0.0	0.12	0.56	0.71
0.19	0.0		
1.59	0.12		

Figure A2. Measured suspended solids levels from biweekly grab samples taken in Little Pine Creek at the upstream (Animal Science Farm) and downstream (Greenhill) sites from 1/1991 to 11/1992.

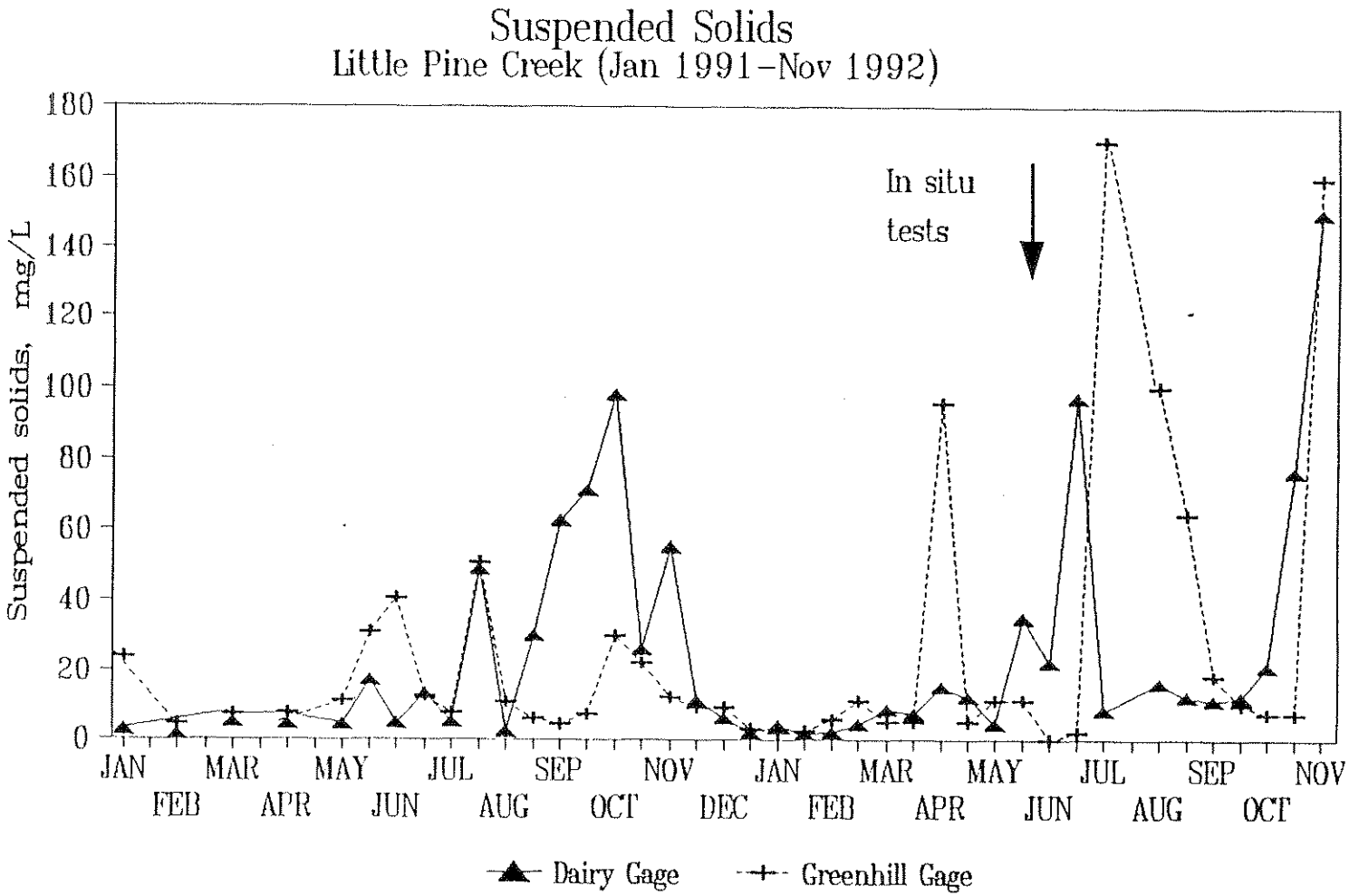
Suspended Solids
Little Pine Creek (Jan 1991–Nov 1992)
Suspended solids, mg/L
180
160
140
120
100
80
60
40
20
0
In situ
tests
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
Dairy Gage
Greenhill Gage

Figure A2.