# Sensor data as a measure of native freshwater mussel impact on nitrate formation and food digestion in continuous-flow mesocosms

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**Abstract:** Native freshwater mussels can influence the aquatic N cycle, but the mechanisms and magnitude of this effect are not fully understood. We assessed the effects of *Amblema plicata* and *Lampsilis cardium* on N transformations over 72 d in 4 continuous-flow mesocosms, with 2 replicates of 2 treatments (mesocosms with and without mussels), equipped with electronic water-chemistry sensors. We compared sensor data to discrete sample data to assess the effect of additional sensor measurements on the ability to detect mussel-related effects on $NO_3^-$ formation. Analysis of 624 sensor-based data points detected a nearly 6% increase in $NO_3^-$ concentration in overlying water of mesocosms with mussels relative to mesocosms without mussels ($p < 0.05$), whereas analysis of 36 discrete samples showed no statistical difference in $NO_3^-$ between treatments. Mussels also significantly increased $NO_2^-$ concentrations in the overlying water, but no significant difference in total N was observed. We used the sensor data for phytoplankton-N and $NH_4^+$ to infer that digestion times in mussels were 13 ± 6 h. The results suggest that rapid increases in phytoplankton-N levels in the overlying water can lead to decreased lag times between phytoplankton-N and $NH_4^+$ maxima. This result indicates that mussels may adjust their digestion rates in response to increased levels of food. The adjustment in digestion time suggests that mussels have a strong response to food availability that can disrupt typical circadian rhythms. Use of sensor data to measure directly and to infer mussel effects on aquatic N transformations at the mesocosm scale could be useful at larger scales in the future.

**Key words:** native freshwater mussels, electronic sensor data, water chemistry, nitrate, inferred digestion time

Native freshwater mussels (Bivalvia:Unionoida; hereafter mussels) are a guild of benthic, burrowing, suspension-feeding bivalves. Mussels are large (25–200+ mm in length) and long-lived (usually >25 y) invertebrates that often are referred to as ecosystem engineers because of their ability to transfer nutrients from the overlying water to the sediments and to stimulate production across multiple trophic levels (Christian et al. 2005, Vaughn et al. 2008). The biomass of healthy mussel beds can exceed that of all benthic organisms by an order of magnitude (Negus 1966, Layzer et al. 1993), and production by mussels can equal that of all other macrobenthos in many rivers (Strayer et al. 1994, Vaughn et al. 2004). As a consequence, mussels can influence a variety of biogeochemical cycles in waters they inhabit. *Amblema plicata* and *Lampsilis cardium* are mussels of interest for N-cycle studies because they make up a large fraction of the mussel biomass in the Iowa River (Poole and Downing 2004) and the Upper Mississippi River watershed (Zohrer 2006, Newton et al. 2011), which are plagued with N management issues (Goolsby et al. 2001).

Mussels influence the aquatic N cycle by filtering phytoplankton and other N-containing particulate organic matter from the overlying water (Kaspar et al. 1985, Prins and Smaal 1994, Thorp et al. 1998). Some captured N is used for growth, and the remainder is released for transformation or assimilation by bacteria and other organisms (Vaughn et al. 2004). $NH_4^+$ excretion and biodeposition of feces and pseudofeces provide N to the benthic zone (Gardner et al. 2001, Howard and Cuffey 2006). Excretion also increases $NH_4^+$ concentrations in the overlying water near mussel beds (Vaughn et al. 2008). In adequately oxygenated sediment and overlying water, $NH_4^+$-oxidizing and $NO_2^-$-oxidizing bacteria convert significant quantities of $NH_4^+$ to $NO_3^-$ (Watson et al. 1989, Jones et al. 1995,

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Strauss et al. 2004, Canfield et al. 2010). $NO_3^-$ is the least reactive and most readily transported form of aquatic N in aerobic main channels of streams and rivers. Excess $NO_3^-$ contributes to harmful algal blooms and to hypoxia in the Gulf of Mexico. Therefore, the influence of mussels on phytoplankton, $NH_4^+$, $NO_3^-$, and other N constituents in aquatic systems must be understood more fully.

The collective effect of mussels on the aquatic N cycle is the culmination of short-term influences over time (Christian et al. 2008). Food requirements and availability influence feeding rhythms of freshwater (Englund and Heino 1994b, Hwang et al. 2004, Haag 2012) and marine mussels (Robson et al. 2010). Mussels display a diurnal rhythm of valve gaping with indications (greater gape angle, increased pumping rate, and increased respiration rate) of elevated nighttime activity possibly associated with feeding (McCorkle et al. 1979, Jorgensen et al. 1988, Englund and Heino 1994a, Mulholland et al. 2006). The influence of short-term disruptions on rhythmic feeding cycles of mussels limits our ability to adequately describe or predict seasonal and longer-term effects of mussels on the aquatic N cycle.

Recent advances in electronic sensing have enabled data collection at increasingly shorter time scales in a variety of aquatic systems (Sandford et al. 2007, Pellerin et al. 2009, Bril 2010, Loperfido et al. 2010). These data-collection advances often provide opportunity to develop and test new or refined hypotheses. We coupled 30-min data for chlorophyll *a* (chl *a*, a surrogate measure of phytoplankton), $NH_4^+$, and $NO_3^-$ from submerged electronic sensors with conventional 3-times-daily discrete sampling measurements of $NH_4^+$, $NO_3^-$, and additional constituents in laboratory mesocosms. This experimental design enabled us to test hypotheses regarding short-term aquatic N transformations while providing necessary quality-control data for the sensor measurements. For example, we tested the hypothesis that mussels would significantly increase concentrations of $NO_3^-$ in the overlying water. This hypothesis is difficult to test in $NO_3^-$-rich river waters where mussels affect a relatively small change on a $NO_3^-$ mass basis. In addition, the sensor data for phytoplankton-N and $NH_4^+$ informed a potentially new approach to inferring phytoplankton digestion time in mussels. Our study is an example of how advances in data collection can influence the formation of new hypotheses. Our results show that sensor data can enhance and complement data collected by traditional methods toward the pursuit of further understanding the effects of freshwater mussels on aquatic systems.

## METHODS

We conducted our experiment for 72 d (8 May–19 July 2012). It culminated in a 7-d intensive analysis period from 13 July to 19 July 2012 (experimental days 65–72). We used flow-through mesocosms (61 × 61 × 61 cm) each containing 140 L of continuously fed, untreated Iowa River water with an 8-cm-thick bottom sand layer that accumulated river sediment over time (Fig. 1). Two mesocosms contained mussels from the Iowa River and 2 contained no mussels (control). We placed 12 *Amblema plicata* (95 ± 20 mm) and 13 *Lampsilis cardium* (120 ± 25 mm) in one mesocosm (70 mussels/$m^2$) and 13 *A. plicata* and 12 *L. cardium* (with similar length distributions) in another. The gravity-fed, constant-head (415-L head tank) system provided a mean flow rate of ~55 L/h, resulting in a mean hydraulic retention time of ~2.5 h/mesocosm. We used custom-built flow measurement devices (tipping buckets) with magnetic reed switches to quantify influent flow. Complete mixing in each mesocosm was provided by 1500 L/h submersible pumps (model EcoPlus Eco 396 Submersible Pump; Sunlight Supply, Inc., Vancouver, Washington), and two 1000-W solar simulators (model Phyto-Lite I Series; Sunlight Supply, Inc., Vancouver, Washington) provided illumination on a 12:12 h light-dark cycle. Photosynthetically active radiation (PAR) sensors measured solar irradiance at the substrate (model SQ-120; Apogee Instruments, Logan, Utah) and water surface (model LI190SB-L; Campbell Scientific, Logan, Utah) of each mesocosm. All sensor measurements were collected using 2 data loggers (model CR1000; Campbell Scientific).

### Sensor-based water-chemistry measurements

We used electronic water-chemistry sensors, arranged on a commercially available multisensor device (model DS5, Hach Chemical Company, Loveland, Colorado), to measure water-chemistry variables every 30 min in the overlying water of each mesocosm and the head tank. The water-chemistry sensors measured chl *a* (compact fluorometer), $NH_4^+$ and $NO_3^-$ (ion-selective electrodes), dissolved $O_2$ (luminescent dissolved $O_2$), temperature, and pH. We calibrated the sensors according to manufacturer specifications with Hydras 3 LT software (Hach Chemical Company, Loveland, Colorado) and certified reagents (Fisher Scientific, Pittsburgh, Pennsylvania). We calibrated the chl *a* sensor with a 200 $\mu$g/L certified standard (Turner Designs, Sunnyvale, California). We normalized chl *a*, $NH_4^+$, and $NO_3^-$ sensor data in a manner analogous to the internal standard method (USEPA 2010) with discrete sample results as calibration points.

### Discrete water-chemistry measurements

We collected overlying water samples daily at 0800, 1000, and 1600 h throughout the 7-d intensive discrete sampling period. We collected these samples in small plastic containers and analyzed chl *a*, $NH_4^+$, $NO_3^-$, $NO_2^-$, and total N. We measured chl *a* by fluorescence, $NH_4^+$ by the salicylate method, $NO_3^-$ by the dimethylphenol method, and $NO_2^-$ by the diazotization method (CFR 2012). We

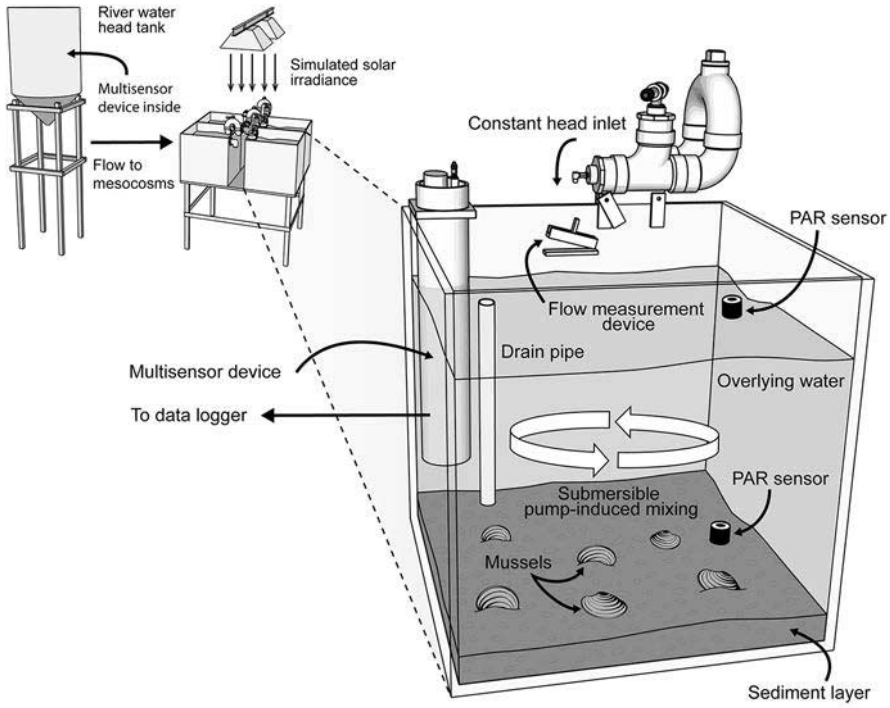


Figure 1. Schematic diagram of the flow-through, 4-mesocosm system, which was continuously fed Iowa River water (monitored with a multisensor device), contained a sand and river-sediment bottom layer, and was irradiated with simulated sunlight (12 h daily). Each mesocosm was equipped with a constant head inlet, a flow measurement device, a recirculating pump, photosynthetically active radiation (PAR) sensors, and a multisensor, water-chemistry device. Two mesocosms contained mussels, and 2 contained no mussels.

measured total N by persulfate digestion method 4500-N C (APHA 2012). To facilitate direct comparison of all N-containing species, we converted measured chl *a* concentrations (μg/L) to phytoplankton biomass as N (phytoplankton-N, mg N/L) based on literature values (Kasprzak et al. 2008) and the empirical formula $C_{106}H_{263}O_{110}N_{16}P$ (Chapra 1997). Two overlying water samples were analyzed by the State Hygienic Laboratory at the University of Iowa (Coralville, Iowa) for phytoplankton taxa based on microscopic examination and counting method 10200 (APHA 2012). We collected porewater samples by syringe daily at 0800, 1000, and 1600 h during the 7-d intensive period and analyzed $NH_4^+$, $NO_3^-$, and $NO_2^-$. We analyzed porewater total organic C (TOC) at the beginning and end of the experiment with direct method 415.3 (CFR 2012).

### Sensor-based estimation of digestion time

We analyzed the sensor data for phytoplankton-N and $NH_4^+$ to investigate their potential use as surrogate indicators of phytoplankton-N digestion time in mussels. We assumed that increases in $NH_4^+$ maxima in the mussel treatment that exceeded increases related to bacterial activity in the no-mussel treatment were the result of digestion of phytoplankton-N by mussels. We used the lag time between 5 phytoplankton-N maxima and subsequent $NH_4^+$ maxima from the 7-d intensive sampling period to estimate the *inferred digestion time* attributable to mussels.

### Water-chemistry data quality assurance

We analyzed a reagent blank (deionized water) and certified standard solutions with each discrete water-sample batch and used a rejection criterion of ±10% from expected values. We used standard concentrations of 1 mg $NH_4^+$/L, 10 mg $NO_3^-$/L, 1 mg $NO_2^-$/L, 10 mg N/L, and 20 mg C/L (Hach Chemical Company, Loveland, Colorado). We tested the accuracy of the chl *a* measurements with 12 and 80 $\mu$g chl *a*/L stock solution (Turner Designs, Sunnyvale, California).

### Water-chemistry calculations and statistical analysis

We used the trapezoidal rule to convert phytoplankton-N, $NH_4^+$, $NO_3^-$, $NO_2^-$, and total N concentration-vs-time data from the 7-d intensive analysis period to mass measurements (SigmaPlot, version 12.0; Systat Software, San Jose, California). We calculated the mass difference of each N species by subtracting the total mass of mean, replicate data in the control treatments from the total mass of mean, replicate measurements in the mussel treatments.

We used the Mann–Whitney Rank Sum test to estimate the effects of mussels on N dynamics relative to control treatments for the discrete data from the overlying water and pore water. We used analysis of covariance (ANCOVA) to estimate the effects of mussels for the overlying water-sensor data. We used a sum-of-squares adjustment to account for nonorthogonal elements in the experimental design; we analyzed simulated solar irradiance, water flow, and water temperature as covariates of the sensor-based treatment data for phytoplankton-N, $NH_4^+$, and $NO_3^-$. We did statistical analyses in Minitab (version 16; Minitab, State College, Pennsylvania).

## RESULTS

Across all 4 mesocosms, water temperature ranged from 23 to 28°C (mean = 25°C). Mean dissolved $O_2$ concentration and pH were 6.8 ± 0.74 mg/L and 8.2 ± 0.12 in the controls and 5.9 ± 0.71 mg/L and 7.9 ± 0.08 in the mussel treatments, respectively. Irradiance was ~730 μmol $m^{-2}$ $s^{-1}$ at the water surface and ~40 μmol $m^{-2}$ $s^{-1}$ at the substrate during solar simulator operation. The mean phytoplankton biomass in the mesocosms was 2.5 mg/L (0.16 mg N/L) on days 65 and 72. Diatoms were the most dominant taxon present (50%), followed by Cyanobacteria (19%), Chlorophyta (18%), and Protozoa (11%). The mesocosm Reynolds number, an estimate of near-bed turbulence, was $4.2 \times 10^5$.

### Water chemistry

The overlying-water sensor data collected for each duplicate treatment during the 7-d intensive analysis period were combined to provide 624 measurements each for the control and mussel treatments. The sensor data and the corresponding discrete data set represent the collective water-chemistry changes over the entire 72 d experiment. The comparatively large sensor-enabled data set showed significant differences in mean phytoplankton-N, $NH_4^+$, and $NO_3^-$ between treatments (Table 1). Solar irradiance was a significant source of variation for sensor-based phytoplankton-N, $NH_4^+$, and $NO_3^-$ measurements ($p < 0.05$; Table 2). Water flow was a significant source of variation in phytoplankton-N, $NH_4^+$, and $NO_3^-$ measurements, but the variation was relatively small (e.g., phytoplankton-N $F = 151$ for flow vs 775 for treatment). Water temperature was a major source of variability ($F = 1757$ for temperature vs 222 for treatment) in the sensor-based $NO_3^-$ data. However, this variability did not impede measurement of a significant but relatively small increase in $NO_3^-$ concentration caused by mussels (Fig. 2). The much smaller ($n = 36$) discrete data set from the overlying water also revealed significant differences between treatments in phytoplankton-N and $NH_4^+$, but not $NO_3^-$ (Fig. 3A, Table 1). Overlying-water discrete data means for $NO_2^-$ were statistically different between treatments, but the difference in total N was not significant. Mean

Table 1. Concentrations, % differences between treatments, mass differences between treatments, and *p*-values for water-quality measurements from sensors and discrete sampling in mesocosms with and without mussels (treatments). Concentrations are mg N/L for all N species and mg C/L for total organic C (TOC).

| | Treatment (mg/L) | | | | Difference | | |
|---|---|---|---|---|---|---|---|
| | Control | | Mussel | | | | |
| Variable | Mean | SD | Mean | SD | % | Mass (mg) | *p* |
| Sensor data, overlying water | | | | | | | |
| Phytoplankton-N | 0.13 | 0.06 | 0.07 | 0.04 | −48 | −531 | <0.001 |
| $NH_4^+$ | 0.05 | 0.01 | 0.09 | 0.01 | 96 | 396 | <0.001 |
| $NO_3^-$ | 0.58 | 0.11 | 0.62 | 0.12 | 5.9 | 300 | <0.001 |
| Discrete data, overlying water | | | | | | | |
| Phytoplankton-N | 0.14 | 0.06 | 0.07 | 0.03 | −47 | −547 | <0.001 |
| $NH_4^+$ | 0.06 | 0.02 | 0.09 | 0.02 | 66 | 312 | <0.001 |
| $NO_3^-$ | 0.57 | 0.12 | 0.61 | 0.14 | 5.7 | 267 | 0.193 |
| $NO_2^-$ | 0.03 | 0.01 | 0.05 | 0.01 | 39 | 111 | <0.001 |
| Total N | 1.2 | 0.20 | 1.2 | 0.19 | 6.6 | 650 | 0.102 |
| Discrete data, porewater | | | | | | | |
| $NH_4^+$ | 1.0 | 0.37 | 2.6 | 0.71 | 160 | – | <0.001 |
| $NO_3^-$ | 0.29 | 0.12 | 0.40 | 0.17 | 38 | – | 0.008 |
| $NO_2^-$ | 0.04 | 0.02 | 0.06 | 0.03 | 40 | – | 0.031 |
| TOC | 14 | 5.3 | 18 | 2.9 | 26 | – | – |

Table 2. Results of the analysis of covariance (ANCOVA) performed on sensor data to test for differences in phytoplankton-N, $NH_4^+$, and $NO_3^-$ concentrations in mesocosms with and without mussels (treatment). Covariates were solar irradiance, water flow, and temperature.

| Variable | Adjusted Sum of Squares | $F$ | $p$ | $R^2$ (%) |
|---|---|---|---|---|
| Phytoplankton-N | | | | 53.5 |
| Irradiance | 0.016 | 10.4 | 0.001 | |
| Flow | 0.23 | 151 | <0.001 | |
| Temperature | 0.037 | 24.4 | <0.001 | |
| Treatment | 1.2 | 775 | <0.001 | |
| $NH_4^+$ | | | | 95.0 |
| Irradiance | 0.0003 | 10.8 | 0.001 | |
| Flow | 0.0042 | 150 | <0.001 | |
| Temperature | 0.012 | 431 | <0.001 | |
| Treatment | 0.63 | 22,733 | <0.001 | |
| $NO_3^-$ | | | | 84.4 |
| Irradiance | 0.017 | 8.2 | 0.004 | |
| Flow | 0.0096 | 4.6 | 0.031 | |
| Temperature | 3.6 | 1757 | <0.001 | |
| Treatment | 0.45 | 222 | <0.001 | |

$NH_4^+$ concentration in porewater differed between treatments ($p < 0.001$), as did $NO_3^-$ and $NO_2^-$ ($p = 0.008$ and 0.031, respectively; Fig. 3B, Table 1).

### Phytoplankton digestion time

The sensor data showed that consumption by mussels reduced the phytoplankton-N maximum (Fig. 4A) and that mussels increased the $NH_4^+$ maximum (Fig. 4B) relative to mesocosms without mussels. Inferred digestion time was estimated from phytoplankton-N maxima at ~65.9, 66.8, 68.5, 69.3, and 70.3 d and subsequent $NH_4^+$ maxima at ~66.7, 67.6, 68.9, 69.6, and 70.8 d (drop lines in Fig. 4). The inferred digestion times were 18.5 to 20.5 h before a phytoplankton-N spike at 68.5 d and 7 to 12.5 h after the spike, resulting in a mean inferred digestion time of 13 ± 6 h.

## DISCUSSION

Our use of Iowa River water and sediments in laboratory mesocosms provided habitat conditions, including microbial activity, representative of what mussels in the Iowa River might encounter in the natural environment. Water temperature, pH, and solar irradiance in the mesocosms were similar to typical Iowa River conditions from June through August (Alados et al. 2000, Espinosa-Villegas et al. 2004, Garrett 2012). Typical Iowa River $NO_3^-$ concentrations are between ~1 and 10 mg N/L (mean ≈ 5 mg N/L; Espinosa-Villegas et al. 2004), although the actual concentrations are highly variable. The $NO_3^-$ concentrations in our experiment were uncharacteristically low because of drought conditions in the Iowa River basin at the time of the experiments. Dissolved $O_2$ concentrations in the mesocosms were slightly lower than typical Iowa River values (Espinosa-Villegas et al. 2004), but were well above levels needed by mussels (McMahon 1996, Yu and Culver 1999). Phytoplankton composition was similar to that in the Iowa River (JSB, unpublished data), but biomass concentrations were lower than usual because of the drought. The Reynolds number estimate for the mesocosms was lower than reported for the Mississippi River main channel ($4 \times 10^6$–$8 \times 10^7$; Molinas and Wu 2001), but similar to that experienced by mussels with a preference for main-channel border areas and small side channels (Zigler et al. 2008).

The increased statistical power provided by the sensor data for each treatment ($n = 624$) enabled the ~6% increase in $NO_3^-$ concentration attributable to mussels to be deemed significant. This increased power is a marked improvement in our ability to assess the effect of mussels on $NO_3^-$ concentrations, particularly in $NO_3^-$-rich waters. However, this result should be interpreted with caution because statistically significant differences in water-chemistry measurements do not necessarily equate to biologically significant differences. In contrast, the 72-d effect of mussels on phytoplankton-N and $NH_4^+$ could be detected with less-frequent, discrete sampling because the magnitude of the % difference between treatments was much greater for these constituents. To our knowledge, we are the first to report a difference in $NO_2^-$ concentrations in overlying water between no-mussel and mussel treatments (0.03 mg N/L vs 0.05 mg N/L, respectively). Our use of sensor data to improve measurability of $NO_3^-$ combined with discrete sampling to measure $NO_2^-$ and other N constituents provided valuable results.

We propose that sensor data for phytoplankton-N (indicated by chl *a* measurements) and $NH_4^+$ might be useful for inferring digestion time in mussels. To our knowledge,

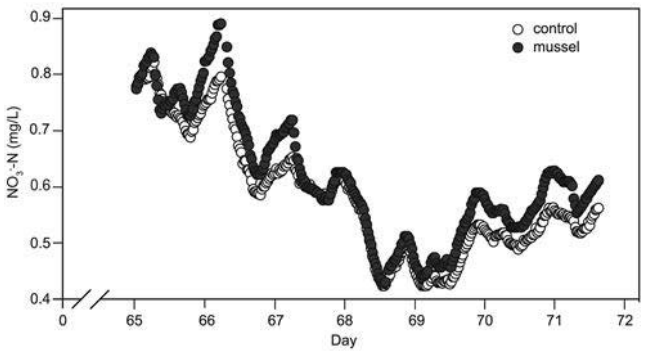


Figure 2. Sensor-based measurements of $NO_3^-$ concentrations in the overlying water during the 7-d intensive sampling period show a nearly 6% increase attributable to mussels.

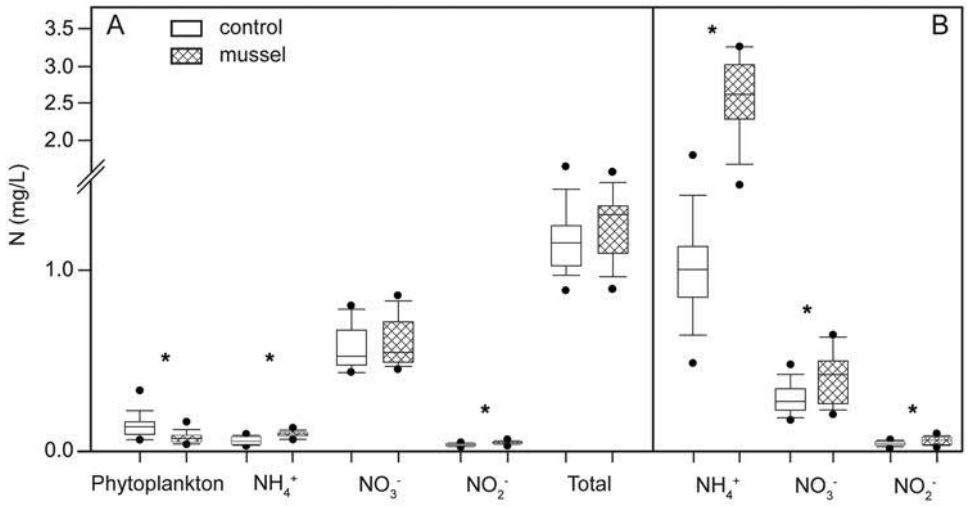


Figure 3. Box-and-whisker plot for discrete data from overlying ($n$ = 36) (A) and porewater ($n$ = 36) (B) for the mesocosms with and without mussels. Lines inside boxes represent means, ends of boxes are the 25$^{th}$ and 75$^{th}$ percentiles, whiskers are the 10$^{th}$ and 90$^{th}$ percentiles, and black dots are outliers. * indicates statistically significant differences between treatments.

most digestion studies have been done with marine mussels fed $^{14}$C-labelled food, and digestion durations were between 1 to 64 h (Bayne et al. 1987, Wang et al. 1995, Wang and Fisher 1996). Our inferred result (13 ± 6 h) is within this reported range. Moreover, the difference in phytoplankton-N concentration between treatments (0.06 mg-N/L; Table 1) detected with sensor data was comparable to the difference in $NH_4^+$ when expressed on an N-equivalent basis (0.04 mg N/L; Table 1). This result provides further evidence that phytoplankton-N losses (digestion) are coupled with $NH_4^+$ gains particularly because they were measured with a sensitive method (electronic sensing) in a system where the effects could be attributed specifically to mussels.

Our results suggest that digestion time in mussels is variable and is associated with changing food concentration as has been observed in marine mussels (Bayne et al. 1989, Wang et al. 1995). The increase in phytoplankton-N consumption and the decrease in inferred digestion time following a phytoplankton-N spike demonstrate the ability of mussels to adjust the volume of material held within their digestive systems (Bayne et al. 1987). Efficiencies in food absorption by marine mussels are relatively constant, indicating mussels maintain a balance between total digestive system content and production of digestive enzymes (Bayne et al. 1987). Thus, we expected an increase in food consumption to lead to increased production of digestive enzymes and subsequent decreased digestion time. We are unaware of similar studies on digestion times in freshwater mussels.

Our study shows the value of sensor data as a tool for measuring the effects of native mussels on $NO_3^-$ forma-

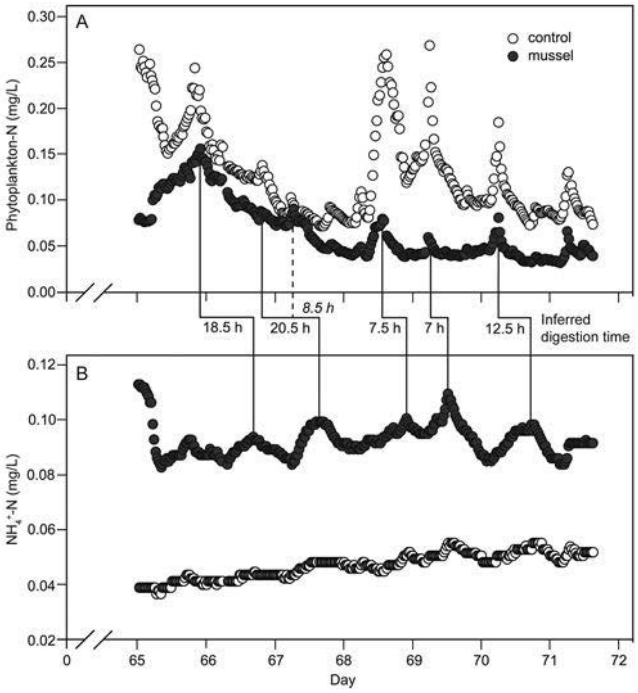


Figure 4. Sensor-based data for phytoplankton-N (A) and $NH_4^+$ (B) concentrations used to infer digestion time in mussels. Each point represents the mean of 2 measurements in the mussel or no-mussel mesocosms. Drop lines connect phytoplankton-N maxima with subsequent $NH_4^+$ maxima with lag times between maxima shown in hours. The dashed drop line indicates an alternative phytoplankton-N maxima to $NH_4^+$ maxima lag time that arguably could have been chosen.

tion in continuous-flow mesocosms. Our results are congruent with those of previous studies that have shown the importance of native mussels in N cycling in aquatic systems (e.g., Thorp et al. 1998, Vaughn et al. 2004, 2008). The inference of digestion time in mussels is a promising potential application for sensor data, but further research is needed to associate these inferred times conclusively with directly measured digestion times and to evaluate how microbes might influence this process.

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## LITERATURE CITED

Alados, I., F. J. Olmo, I. Foyo-Moren, and L. Alados-Arboledas. 2000. Estimation of photosynthetically active radiation under cloudy conditions. Agricultural and Forest Meteorology 102: 39–50.

APHA (American Public Health Association). 2012. Standard methods for the examination of water and wastewater. 22nd edition. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

Bayne, B. L., A. J. S. Hawkins, and E. Navarro. 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. Journal of Experimental Marine Biology and Ecology 111:1–22.

Bayne, B. L., A. J. S. Hawkins, E. Navarro, and I. P. Iglesias. 1989. Effects of seston concentration on feeding, digestion, and growth in the mussel *Mytilus edulis*. Marine Ecology Progress Series 55:47–54.

Bril, J. S. 2010. Measuring mussel behavior and analyzing high frequency nitrate data to explore new phenomena in dynamic nutrient cycling. MS Thesis, University of Iowa, Iowa City, Iowa.

Canfield, D. E., A. N. Glazer, and P. G. Falkowski. 2010. The evolution and future of Earth's nitrogen cycle. Science 330: 192–196.

CFR (Code of Federal Regulations). 2012. Title 40—Protection of Environment, parts 136–149. US Government Printing Office, Washington, DC.

Chapra, S. C. 1997. Surface water-quality modeling. Waveland Press, Long Grove, Illinois.

Christian, A. D., B. G. Crump, and D. J. Berg. 2008. Nutrient release and ecological stoichiometry of freshwater mussels (Mollusca: Unionidae) in 2 small, regionally distinct streams. Journal of the North American Benthological Society 27: 440–450.

Christian, A. D., J. L. Harris, W. R. Posey, J. F. Hockmuth, and G. L. Harp. 2005. Freshwater mussel (Bivalvia: Unionidae) assemblages of the Lower Cache River, Arkansas. Southeastern Naturalist 4:487–512.

Englund, V., and M. Heino. 1994a. Valve movement of *Anodonta anatina* and *Unio tumidus* (Bivalvia, Unionidae) in a eutrophic lake. Annales Zoologici Fennici 31:257–262.

Englund, V., and M. Heino. 1994b. Valve movement of freshwater mussel *Anodonta anatina*: a reciprocal transplant experiment between two lakes. Annales Zoologici Fennici 31: 417–423.

Espinosa-Villegas, C., C. L. Just, T. Nakato, and J. L. Schnoor. 2004. Coralville Reservoir water quality project. Final report to the U.S. Army Corps of Engineers, University of Iowa, Iowa City, Iowa. (http://www.iihr.uiowa.edu/wp-content/uploads/2013/06/Coralville-2005-yearly-report.pdf)

Gardner, W. S., L. Y. Yang, J. B. Cotner, T. H. Johengen, and P. J. Lavrentyev. 2001. Nitrogen dynamics in sandy freshwater sediments (Saginaw Bay, Lake Huron). Journal of Great Lakes Research 27:84–97.

Garrett, J. D. 2012. Concentrations, loads, and yields of select constituents from major tributaries of the Mississippi and Missouri Rivers in Iowa, water years 2004–2008. U.S. Geological Survey Scientific Investigations Report 2012-5240: 1–61. US Geological Survey, Reston, Virginia.

Goolsby, D. A., W. A. Battaglin, B. T. Aulenbach, and R. P. Hooper. 2001. Nitrogen input to the Gulf of Mexico. Journal of Environmental Quality 30:329–336.

Haag, W. R. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press, New York.

Howard, J. K., and K. M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460–474.

Hwang, S. J., H. S. Kim, J. K. Shin, J. M. Oh, and D. S. Kong. 2004. Grazing effects of a freshwater bivalve (*Corbicula leana* Prime) and large zooplankton on phytoplankton communities in two Korean lakes. Hydrobiologia 515:161–179.

Jones, J. B., S. G. Fisher, and N. B. Grimm. 1995. Nitrification in the hyporheic zone of a desert stream ecosystem. Journal of the North American Benthological Society 14:249–258.

Jorgensen, C. B., P. S. Larsen, F. Mohlenberg, and H. U. Riisgard. 1988. The mussel pump: properties and modeling. Marine Ecology Progress Series 45:205–216.

Kaspar, H. F., P. A. Gillespie, I. C. Boyer, and A. L. Mackenzie. 1985. Effects of mussel aquaculture on the nitrogen-cycle and benthic communities in Kenepuru Sound, Marlborough Sounds, New Zealand. Marine Biology 85:127–136.

Kasprzak, P., J. Padisak, R. Koschel, L. Krienitz, and F. Gervais. 2008. Chlorophyll *a* concentration across a trophic gradient of lakes: an estimator of phytoplankton biomass? Limnologica 38:327–338.

Layzer, J. B., M. E. Gordon, and R. M. Anderson. 1993. Mussels: the forgotten fauna of regulated rivers: a case study of

the Caney Fork River. Regulated Rivers Research and Management 8:63–71.

Loperfido, J. V., C. L. Just, A. N. Papanicolaou, and J. L. Schnoor. 2010. In situ sensing to understand diel turbidity cycles, suspended solids, and nutrient transport in Clear Creek, Iowa. Water Resources Research 46:1–8.

McCorkle, S., T. C. Shirley, and T. H. Dietz. 1979. Rhythms of activity and oxygen-consumption in the common pond clam, *Ligumia subrostrata* (Say). Canadian Journal of Zoology 57: 1960–1964.

McMahon, R. F. 1996. The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. American Zoologist 36:339–363.

Molinas, A., and B. S. Wu. 2001. Transport of sediment in large sand-bed rivers. Journal of Hydraulic Research 39:373–388.

Mulholland, P. J., S. A. Thomas, H. M. Valett, J. R. Webster, and J. Beaulieu. 2006. Effects of light on $NO_3$-uptake in small forested streams: diurnal and day-to-day variations. Journal of the North American Benthological Society 25:583–595.

Negus, C. L. 1966. A quantitative study of growth and production of unionid mussels in the River Thames at Reading. Journal of Animal Ecology 35:513–521.

Newton, T. J., S. J. Zigler, J. T. Rogala, B. R. Gray, and M. Davis. 2011. Population assessment and potential functional roles of native mussels in the Upper Mississippi River. Aquatic Conservation: Marine and Freshwater Ecosystems 21:122–131.

Pellerin, B. A., B. D. Downing, C. Kendall, R. A. Dahlgren, T. C. Kraus, J. Saraceno, R. Spencer, and B. A. Bergamaschi. 2009. Assessing the sources and magnitude of diurnal nitrate variability in the San Joaquin River (California) with an in situ optical nitrate sensor and dual nitrate isotopes. Freshwater Biology 54:376–387.

Poole, K. E., and J. A. Downing. 2004. Relationship of declining mussel biodiversity to stream-reach and watershed characteristics in an agricultural landscape. Journal of the North American Benthological Society 23:114–125.

Prins, T. C., and A. C. Smaal. 1994. The role of the blue mussel *Mytilus edulis* in the cycling of nutrients in the Oosterschelde estuary (The Netherlands). Hydrobiologia 283:413–429.

Robson, A. A., C. Garcia de Leaniz, R. P. Wilson, and L. G. Halsey. 2010. Effect of anthropogenic feeding regimes on activity rhythms of laboratory mussels exposed to natural light. Hydrobiologia 655:197–204.

Sandford, R. C., A. Exenberger, and P. J. Worsfold. 2007. Nitrogen cycling in natural waters using in situ, reagentless UV spectrophotometry with simultaneous determination of nitrate and nitrite. Environmental Science and Technology 41: 8420–8425.

Strauss, E. A., W. B. Richardson, L. A. Bartsch, J. C. Cavanaugh, D. A. Bruesewitz, H. Imker, J. A. Heinz, and D. M. Soballe. 2004. Nitrification in the Upper Mississippi River: patterns, controls, and contribution to the $NO_3^-$ budget. Journal of the North American Benthological Society 23:1–14.

Strayer, D. L., D. C. Hunter, L. C. Smith, and C. K. Borg. 1994. Distribution, abundance, and roles of freshwater clams (Bivalvia: Unionidae) in the freshwater tidal Hudson River. Freshwater Biology 31:239–248.

Thorp, J. H., M. D. Delong, K. S. Greenwood, and A. F. Casper. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. Oecologia (Berlin) 117:551–563.

USEPA (US Environmental Protection Agency). 2010. Calibration curves: program use/needs. Forum on Environmental Measurements. US Environmental Protection Agency, Washington, DC. (Available from: http://www.epa.gov/fem/pdfs/calibration-guide-ref-final-oct2010.pdf)

Vaughn, C. C., K. B. Gido, and D. E. Spooner. 2004. Ecosystem processes performed by unionid mussels in stream mesocosms: species roles and effects of abundance. Hydrobiologia 527:35–47.

Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409–423.

Wang, W. X., and N. S. Fisher. 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnology and Oceanography 41:197–207.

Wang, W. X., N. S. Fisher, and S. N. Luoma. 1995. Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. Marine Ecology Progress Series 129:165–176.

Watson, S. W., E. Bock, H. Harms, H. P. Koops, and A. B. Hooper. 1989. Nitrifying bacteria. Pages 1808–1834 *in* J. T. Stanley (editor). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, Maryland.

Yu, N., and D. A. Culver. 1999. In situ survival and growth of zebra mussels (*Dreissena polymorpha*) under chronic hypoxia in a stratified lake. Hydrobiologia 392:205–215.

Zigler, S. J., T. J. Newton, J. J. Steuer, M. R. Bartsch, and J. S. Sauer. 2008. Importance of physical and hydraulic characteristics to unionid mussels: a retrospective analysis in a reach of large river. Hydrobiologia 598:343–360.

Zohrer, J. J. 2006. Securing a future for fish and wildlife: a conservation legacy for Iowans. Iowa Department of Natural Resources, Des Moines, Iowa. (Available from: http://www.iowadnr.gov/Environment/WildlifeStewardship/IowaWildlifeActionPlan.aspx)