

Colonial aggregates: effects of spatial position on zebra mussel responses to vertical gradients in interstitial water quality

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Abstract. Vertical gradients in interstitial water quality may develop within densely organized assemblages of sessile aquatic organisms. These gradients may compromise the survival of individuals. We examined whether a vertical gradient of interstitial water chemistry ($\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and dissolved oxygen [DO]) would develop within dense zebra mussel (*Dreissena polymorpha*) colonies in a laboratory flume (flow rate ~ 1 cm/s). Over a 4-h duration, we found that $\text{NO}_3\text{-N}$ concentrations increased, DO decreased, and $\text{NH}_4\text{-N}$ concentrations remained the same from the surface to the base of 6-cm thick zebra mussel colonies. These results were supported by trends found in natural Lake Michigan zebra mussel colonies at 4 to 6 m depths, where $\text{NO}_3\text{-N}$ concentrations at the base of colonies measured 162% of $\text{NO}_3\text{-N}$ concentrations in open water above the colonies. We also examined how vertical water-quality gradients influenced zebra mussel movement and mortality by tracking the vertical position and survivorship of individual zebra mussels in colonies. Substantial movement out of the base of the colony occurred after 7 and 30 d of incubation. After 30 d, 69% of smaller-sized mussels (≤ 6 mm) moved upward within the colony from the base, in contrast to 0% movement of larger mussels (> 20 mm), whose motility may have been impeded. After 30 d, mortality of all size classes significantly increased, with $> 50\%$ mortality occurring in the bottom layer. Our studies suggest that dense colonies produce vertical interstitial water-quality gradients at low flow, and that movement out of the base of the colony by smaller mussels may be an important mechanism for survival in dense colonies.

Key words: colonial aggregations, *Dreissena polymorpha*, dissolved oxygen, interstitial water quality, nitrogen, nitrate, migration, mortality.

Zebra mussels (*Dreissena polymorpha*) usually occur in colonies that can reach densities $> 200,000/\text{m}^2$ (MacIsaac et al. 1991, Dermott et al. 1993). Unlike other mussels and barnacles, zebra mussel colonies are 3-dimensional and can attain a vertical thickness of up to 20–30 individuals (JEM and NCT, unpublished observations). Vertical gradients of dissolved oxygen (DO) and nitrogenous wastes ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) may develop in the water contained within zebra mussel colonies (henceforth referred to as interstitial water) as a result of the cumulative metabolic activities of the mussels. Water near the top of the colony may be renewed more frequently and waste from the mussels may collect at the base of the colony, leading to poorer interstitial water-quality conditions within the

bottom portion of the colonies. Similar gradients occur in benthic periphyton biofilms (Burkholder et al. 1990, Blenkinsopp et al. 1991, Johnson et al. 1997). However, few studies have examined the response of zebra mussels to adverse conditions that could develop as a result of group-living in dense colonies. Existence in a dense colony alters the immediate environment of individual mussels, so knowledge of intra-colony dynamics is likely to enhance our understanding of how zebra mussels and other colonial organisms survive.

Sessile and semisessile benthic organisms experience both costs and benefits of colonial living, depending on their individual spatial position within the colony. For example, blue mussels (*Mytilus edulis*) at the edge of a dense colony face higher predation rates, yet grow at a rate 50% higher than those positioned near the center of the colony because of higher food availability in the ambient waters (Okamura 1986).

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Food availability at the edges of filter-feeding colonies, including *Mytilus edulis* (Butman et al. 1994), barnacles (Pullen and LaBarbera 1991), and bryozoans (Okamura 1988), exceeds that found within central areas of colonies where closely packed neighbors obstruct flow regimes and compete for food resources. Spatial positional effects within benthic colonies greatly depend on ambient flow conditions. For example, Stevenson (1984) and Stevenson and Glover (1993) described how fast-flowing streams support high densities of benthic algal colonies because of the increased delivery of nutrients to cells located at the base of the biofilm. In contrast, low densities of aggregates are favored in situations where the current is not sufficient to widely distribute resources. Thus, attaining an advantageous position within a dense aggregate, especially under low-flow conditions, may be necessary for the survival of individuals living in densely organized groups.

Species living in dense colonies often demonstrate behavioral responses (such as aggression or movement) to resource gradients that develop within the colony. For example, to secure more resources, blackfly larvae (*Simulium vittatum*) exhibit aggression towards neighbors, forcing them to relocate (Hart 1987, Hart et al. 1991). Similarly, motile diatoms actively migrate upward toward the resource-rich surface of a dense periphyton biofilm in response to lower light and inorganic nutrients at the base of the assemblage (Johnson et al. 1997). Because adult zebra mussels are capable of movement (Oldham 1930, Cawein 1993), we examined whether they may move within dense colonies as a survival mechanism in response to less than optimal interstitial water-quality conditions at the base of dense aggregates.

Our study addressed 2 main hypotheses: 1) the interstitial water within dense zebra mussel colonies would develop vertical gradients in DO and nitrogenous wastes across the colony within a short time, and 2) zebra mussels originally positioned near the base of a dense colony may over time migrate upwards to escape adverse interstitial water-quality conditions. We expected that DO concentrations would be highest near the outside surfaces of the colony because of diffusion inwards from lake water, and lowest towards the base and the center of the colony where it was being metabolically consumed. In addition, we expected zebra mussel nitrogenous

waste compounds (e.g., $\text{NH}_4\text{-N}$ and ammonium oxidized to $\text{NO}_3\text{-N}$) to be more concentrated near the base of the colony than near the surfaces of the colony where diffusion and dilution with lake water occur. We expected that small mussels would have the advantage over larger mussels in moving to the surface of the colony because larger mussels may be hindered physically. We also expected to find the highest rates of mortality among the larger size classes located at the base of the colony. We addressed the 2 hypotheses using both laboratory experiments and in situ studies in Lake Michigan.

Methods

Interstitial water quality within dense zebra mussel colonies

Laboratory experiment.—We tested whether a vertical gradient of interstitial water quality develops within a dense *D. polymorpha* colony over a short period of time. To mimic a naturally dense zebra mussel colony, we constructed open-topped cylindrical chambers (7 cm high, 20 cm in diameter) with sides made of aluminum mesh screening (1.0-mm² aperture) attached to an unglazed ceramic tile base (20 cm × 20 cm) with aquarium-grade silicon sealant. Prior to experimentation, zebra mussels collected from southwestern Lake Michigan were acclimated in an experimental 4000-L flow-through flume (length × width × depth dimensions [m] = 9.75 × 0.91 × 0.79) at the Illinois Natural History Survey's Lake Michigan Biological Station (LMBS) in Zion, Illinois. Unfiltered lake water from nearshore Lake Michigan constantly flowed (rate = 1 cm/s) through the flume and ranged in temperature from 10 to 14°C. Water depth in the flumes was 0.612 m and colonies were placed at a depth of ~0.28 m using concrete cinder blocks. Saturated DO concentrations (10.2–11.0 mg O₂/L) occurred at all times within the flume. We manually mixed the zebra mussels to assure a homogenous representation of size classes within each of the replicate colonies ($n = 4$), and added ~6600 zebra mussels to each chamber to produce a 6-cm thick colony of high density (200,000/m²) as reported by others (MacIsaac et al. 1991). We drained all interstitial water before submersing the chamber into the flume to ensure homogeneity of fresh interstitial water for initial mea-

surements. The chamber was incubated within the flume for 4 h.

We measured 4 colonies on 4 separate days by sampling interstitial water from the center of each colony immediately after it was placed into the flume to determine initial concentrations of $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and DO. We examined whether location within the colony (e.g., proximity to flume water and orientation to flow direction) was related to interstitial water quality by sampling 9 interstitial sites, 3 vertical positions (surface, middle, base) at each of 3 horizontal sites (upstream, midstream, and downstream), within each replicate chamber 4 h after incubation ($n = 4$). At each site, we used a 5-cm hypodermic needle attached to a 60-mL syringe to slowly withdraw 20 mL of interstitial water from the zebra mussel colony. For reference conditions, we collected a water sample 1 m upstream of the colony within the flume. We immediately filtered each sample through a 0.45- μm Millipore membrane filter, placed samples on ice, and then added sulfuric acid to bring the pH to 2.2 for chemical stabilization. We analyzed $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations with a Technicon II Auto-Analyzer using the cadmium reduction method and the automated phenate method, respectively (APHA 1995) and also sampled interstitial DO levels at each site within the colony by inserting a Model 57 mini-YSI probe.

We used repeated measures analysis of variance (RM-ANOVA) to test whether $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, or DO concentrations significantly differed across a vertical or horizontal gradient within the zebra mussel colonies after a 4-h incubation time. We report p -values from Wilks' λ statistics. To meet the assumptions of ANOVA, we transformed the $\text{NH}_4\text{-N}$ data with an inverse function. Untransformed $\text{NO}_3\text{-N}$ and DO data successfully met assumptions. We used pairwise comparisons of least square means to examine statistical differences between specific vertical (surface, middle, base) or horizontal (upstream, midstream, downstream) sites within the colony. All statistics were conducted using SAS/STAT (1999. User's guide, release 8.01 edition, SAS Institute, Cary, North Carolina).

In situ field survey.—We conducted a SCUBA survey at 4 to 6 m depths along vertical faces of a breakwater in southern Lake Michigan (Port of Indiana) to discover if vertical gradients in water quality also existed in dense populations of zebra mussels in natural settings. This site

was blanketed by zebra mussel colonies up to 5-cm thick. We collected paired water samples, one at the colony surface and one at the colony base, from each of 10 colonies located on the vertical facing. Selected colonies occurred at least 1.5 m apart from one another. To sample interstitial water, a SCUBA diver used a syringe as described above and capped each needle after sampling to prevent leakage or contamination. We compared water quality within the zebra mussel colony to ambient Lake Michigan water by collecting a 1-L sample from 2 m above the zebra mussel colonies and analyzing 4 subsamples.

We immediately filtered and chemically stabilized each sample on the boat as previously described, and later analyzed $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations. We used a paired t -test to examine differences in $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ between the surface and base of the zebra mussel colonies ($n = 10$) and an unbalanced, 2-sample t -test to examine differences between the base of the zebra mussel colony ($n = 10$) and subsamples of Lake Michigan water ($n = 4$).

Vertical mortality and movement within a dense zebra mussel colony

Laboratory experiment.—We tracked the position and survivorship of horizontal layers of zebra mussels in assembled colonies for different lengths of time to examine how vertical gradients of water quality in interstitial water influenced zebra mussel mortality and movement. We collected and separated zebra mussels into 3 homogeneously mixed groups, each with 4 size classes (I = ≤ 5 mm, II = 6–13mm, III = 14–20 mm, IV = >20 mm). The 3 groups of mussels were spread on paper towels to allow their shells to air dry for 1 h. The shells of some mussels remained open during the drying process. We then agitated the mussels to induce shell closure and uniformly sprayed each group of mussels with a different color (blue, red, or yellow) of common, nontoxic acrylic paint. We used the same proportion of size-class individuals in each color group for each replicate. The paint then air-dried for another hour before we reintroduced the mussels to the holding flume. Zebra mussels can tolerate extended periods of exposure to air (up to 1 wk) (Ricciardi et al. 1995, McMahon 1996, Paukstis et al. 1999). However, we examined mussels 1 d after spraying to en-

sure that the air-drying and painting did not harm them. Less than 1% of the total number of zebra mussels died after being dried and painted. We removed any mussels with gaping shell openings that did not close with mechanical stimulation.

We placed painted mussels in 3 horizontal layers within 6 cylindrical chambers to quantify mortality and movement. A 2-cm layer of unpainted mussels of random size classes (not tracked) separated the differentially colored layers of painted mussels, producing six, 6-cm thick colonies, each having 5 layers organized from top to bottom as: red, unpainted, blue, unpainted, and yellow mussels. We secured a nylon screen (1-mm mesh) over the top of each chamber to prevent mussels from migrating out of the colony during the experiments. Colonies remained in the LMBS flumes for 7 ($n = 3$) or 30 d ($n = 3$) at flow rates of 1 cm/s.

We took the intact chambers out of the flumes at the end of each incubation period and disassembled them by making 2 horizontal cuts in the colonies, using a 20-cm wide spatula, to carefully separate the 6-cm colonies into 3 layers (i.e., surface 2 cm, center 2 cm, and base 2 cm). The layers were gently separated using a spatula to prevent damage to the mussels. Zebra mussel colonies were loosely connected with byssal threads that could be easily severed. When an individual zebra mussel bisected a horizontal plane between layers, we placed that mussel with the layer in which most of the zebra mussel occurred. No mortality of zebra mussels occurred because we were able to pry apart the loosely connected colonies without cutting through individuals. We then quantified the survivorship and vertical positions of each size class for each colored group of zebra mussels. We analyzed % mortality of zebra mussels of each size class in each layer (surface, middle, or base) after 7 and 30 d of incubation using separate 2-way ANOVAs. We identified significant differences in mortality between layers and size classes using a Tukey's multiple comparison test (MCT).

We also investigated whether zebra mussels migrated within the colony after 7 or 30 d by analyzing the % of individuals that migrated upward from their original position in the middle or base layers for each of the size classes using 2-way ANOVAs. To compensate for any passive movement of mussels that occurred as

a result of handling, we subtracted the number of mussels that were displaced in a control chamber (i.e., lowering a colony into the flume and then immediately removing it) from the overall % that migrated after 7 and 30 d. Handling displaced <1% of all size classes from the base layer, and only 0.6 to 2.7% moved upward from the middle layer, depending on size. For each incubation day, we identified significant differences in upward movement between size classes using a Tukey's MCT.

In situ field survey.—We cored 8 natural zebra mussel colonies (at least 5-cm thick) along the previously described breakwater in southwestern Lake Michigan to test if the abundance and distribution of size classes differed between the base and surface layers of a natural colony. There could be a nonrandom, vertical, size-class distribution within natural colonies if natural vertical gradients in water quality exist, and smaller zebra mussels can more effectively move than larger mussels to escape poor water-quality conditions. We used cylindrical aluminum cores (8.6 cm diameter, 5 cm depth) to collect intact colonies. SCUBA divers firmly pushed the core into the zebra mussel colony until it reached the rock substratum, then slipped a thin steel plate under the core to dislodge mussels from the substratum, and lastly placed pre-cut disks of porous foam padding (8 cm diameter) over the surface of the cored colony to prevent movement within the corer. Plastic lids sealed each end of the core.

Cores were transferred to shore and separated into 2 layers: the bottom 2 cm, and the remaining upper layer. We separated the mussels within each layer into 4 different size classes (as defined above) and enumerated living and dead individuals within each size class. We examined the % of different sizes that occurred in the surface layer and bottom layers using a 2×4 contingency table and χ^2 statistics.

Results

Interstitial water quality within dense zebra mussel colonies

Laboratory experiment.—Interstitial $\text{NO}_3\text{-N}$ concentration within the colonies was initially 0.33 (± 0.02 SE) mg/L, and increased significantly after 4 h of incubation (RM-ANOVA, time $F_{1,27} = 288.04$, $p = 0.0001$). Nitrate increased signifi-

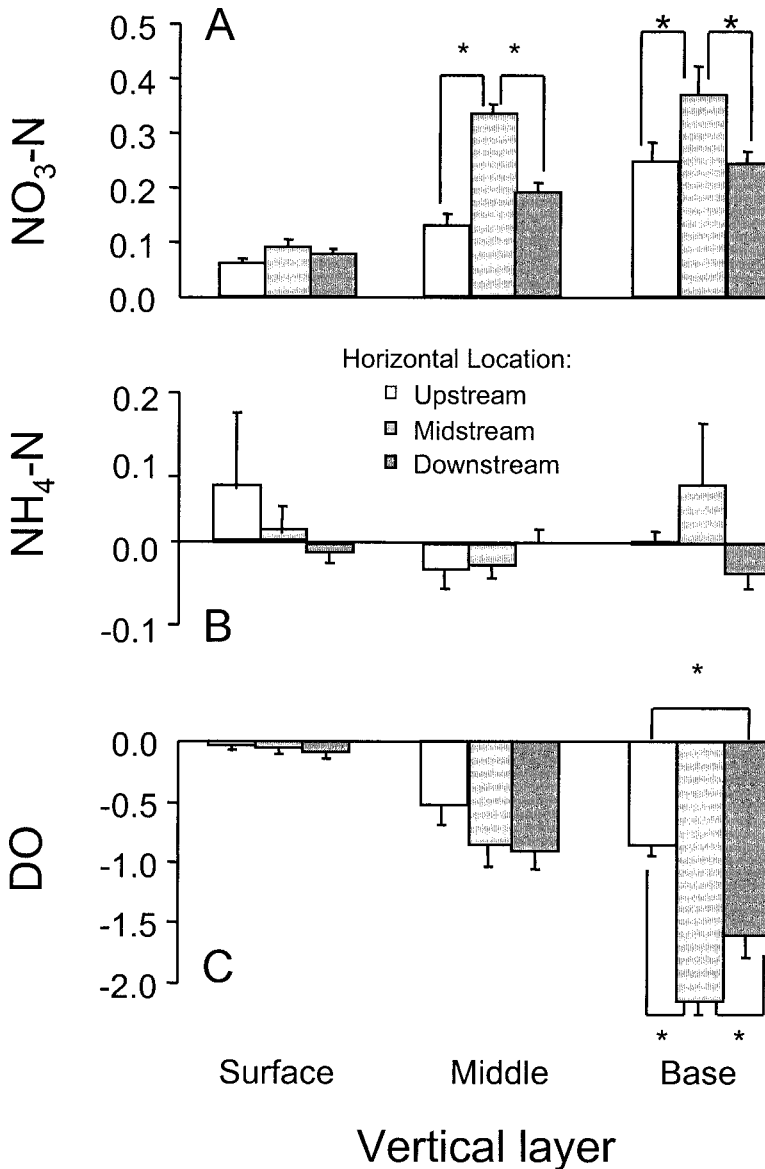


FIG. 1. Change in interstitial water-quality parameters relative to initial concentrations: (A) $\text{NO}_3\text{-N}$, (B) $\text{NH}_4\text{-N}$, and (C) dissolved oxygen (DO) (all $\text{mg/L} + 1 \text{ SE}$) at different horizontal locations (upstream, midstream, and downstream) within vertical layers of a constructed zebra mussel colony (surface, middle, and base layers) over 4 h at low-flow conditions. Note different scales on the y-axes. Brackets show strong statistical differences between horizontal sites, and p -values result from post-hoc pairwise comparison tests. * = $p < 0.05$.

cantly over time (4 h) with vertical depth into the zebra mussel colonies (Fig. 1A; RM-ANOVA, $F_{2,227} = 29.72$, $p = 0.0001$). Nitrate in the surface layer of the colony increased by an average of 0.076 mg/L (i.e., 23%) above initial concentrations, whereas $\text{NO}_3\text{-N}$ in the base layer in-

creased 0.289 mg/L (88%) above initial concentrations. Nitrate concentrations over time also significantly differed between horizontal locations within the colony (Fig. 1A; RM-ANOVA, $F_{2,227} = 10.07$, $p = 0.0005$). No significant interaction occurred between horizontal and vertical

position over time (Fig. 1A; RM-ANOVA, $F_{4,27} = 1.93$, $p = 0.13$), although other patterns in concentrations appeared.

The greatest $\text{NO}_3\text{-N}$ concentrations occurred in the midstream locations for all 3 vertical layers. These areas were furthest away from the margins of the colony. In the surface layer, $\text{NO}_3\text{-N}$ in the midstream location was 0.091 mg/L compared to 0.061 mg/L in the upstream location and 0.078 mg/L in the downstream location. The increase in $\text{NO}_3\text{-N}$ in the midstream location (0.336 mg/L = 101%) of the middle layer significantly exceeded the increases in either upstream (0.131 mg/L = 40%) or downstream (0.190 mg/L = 58%) locations ($p = 0.0003$ and $p = 0.006$, respectively). Significant increases in $\text{NO}_3\text{-N}$ also occurred in the midstream location (0.371 mg/L = 113%) compared to upstream (0.250 mg/L = 76%) and downstream (0.245 mg/L = 74%) locations of the base layer (Fig. 1A; $p = 0.019$, $p = 0.015$, respectively).

Interstitial $\text{NH}_4\text{-N}$ concentrations were initially 0.084 (± 0.02) mg/L. Ammonia concentrations changed significantly over the 4 h incubation (Fig. 1B; RM-ANOVA, time $F_{1,27} = 19.64$, $p = 0.0001$), but not in any clear direction. In contrast to $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ concentrations at different vertical (RM-ANOVA, $F_{2,27} = 0.18$, $p = 0.84$) and horizontal locations (RM-ANOVA, $F_{2,27} = 0.48$, $p = 0.62$) within the zebra mussel colony did not change significantly over time and failed to demonstrate discernable trends (Fig. 1B). In addition, no significant interactions occurred between horizontal and vertical locations over time (Fig. 1B; RM-ANOVA, $F_{4,27} = 0.35$, $p = 0.84$).

Dissolved oxygen changed significantly over time (Fig. 1C; RM-ANOVA, $F_{1,27} = 276.41$, $p = 0.0001$) from its initial concentration of 10.65 (± 0.08) mg/L. Dissolved oxygen decreased significantly with vertical depth into the zebra mussel colonies (Fig. 1C; RM-ANOVA, $F_{2,27} = 83.24$, $p = 0.0001$). The base layer of zebra mussels experienced a significantly larger loss (average of 1.5 mg/L = 14% total) in DO than the middle (0.76 mg/L = 7%) or surface layers (0.05 mg/L \leq 1%) ($p = 0.0001$). In addition, we found significant differences in DO concentrations along the upstream–downstream gradient (RM-ANOVA, $F_{2,27} = 12.12$, $p = 0.0002$). The midstream location in the base layer experienced a 20% decrease in DO (2.15 mg/L), which was a significantly greater reduction than occurred in

either the upstream (0.85 mg/L = 8%) or downstream (1.6 mg/L = 15%) locations in the base layer ($p = 0.0001$ and $p = 0.0008$, respectively). Although there was a significant horizontal effect in the base layer, no significant differences occurred between horizontal positions within the surface or middle vertical layers. A significant interaction among vertical and horizontal positions also occurred over time ($F_{4,27} = 5.73$, $p = 0.0018$), possibly because of strong horizontal trends within the base layer (Fig. 1C). Dissolved oxygen declined a maximum of 20% from its initial concentration, but anaerobic levels were never reached (lowest DO measurement = 6.0 mg/L).

In situ field survey.—Lower concentrations of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ occurred within in situ field colonies (ranges for $\text{NO}_3\text{-N}$ = 0.29–0.31 and $\text{NH}_4\text{-N}$ = 0.09–0.31 mg/L) than in the laboratory colonies (ranges for $\text{NO}_3\text{-N}$ = 0.33–0.75 and $\text{NH}_4\text{-N}$ = 0.02–5.5 mg/L). No significant differences in either $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ existed between the surface and the base vertical layers of the Lake Michigan colonies (Fig. 2A; $\text{NO}_3\text{-N}$, t -test, $t = 1.472$, $p = 0.18$; $\text{NH}_4\text{-N}$, t -test, $t = 0.895$, $p = 0.39$). However, we found significantly higher (162%) $\text{NO}_3\text{-N}$ levels within the interstitial water drawn from the base of the in situ zebra mussel colonies compared to Lake Michigan water (Fig. 2B; t -test, $t = 15.8$, $p = 0.001$). We were unable to measure in situ DO concentrations. Ammonia concentrations tended to be higher in the zebra mussel colonies than in Lake Michigan water, but measurements were highly variable and did not differ significantly (Fig. 2B; t -test, $t = 0.369$, $p = 0.72$).

Vertical mortality and movement within a dense zebra mussel colony

Laboratory experiment.—Mortality of all size classes was significantly higher in the base (>50%) than the surface layers (<1%) (Fig. 3; 2-way ANOVA, $F_{2,24} = 262.6$, $p = 0.0001$) after 30 d of incubation at low flow. Mortality at the base of the colony significantly exceeded the mortality in the middle layer, which was also significantly greater than mortality at the surface (Tukey's MCT, $p < 0.05$). In contrast to our expectations, we did not detect differential mortality among size classes (Fig. 3; 2-way ANOVA, $F_{3,24} = 0.54$, $p = 0.66$), and no significant interaction occurred between layer and size class (2-way

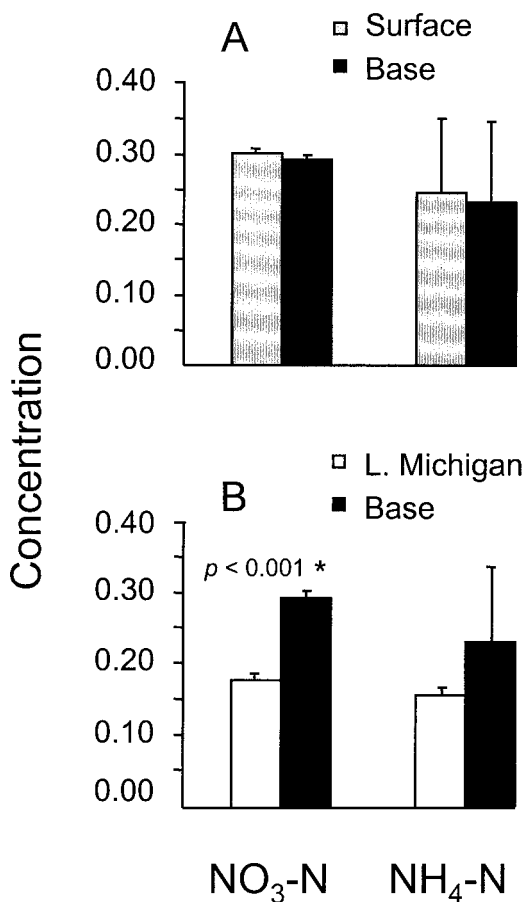


FIG. 2. In situ concentrations (mg/L + 1 SE) of NO₃-N and NH₄-N averaged from 10 zebra mussel colonies occurring in southwestern Lake (L.) Michigan. A.—Concentrations between the surface and base of the zebra mussel colonies. B.—Interstitial concentrations from within the colonies compared to L. Michigan conditions.

ANOVA, $F_{6,24} = 0.83$, $p = 0.56$). Mortality patterns among size classes after 7 d were similar to the 30 d results, but the 7-d data failed to meet normality assumptions of ANOVA and could not be successfully transformed. Therefore, we only report the results for the 30 d treatment (Fig. 3).

We examined vertical movement upwards from the middle and base layers by each size class in these same colonies. The % of total individuals (all size classes combined) moving upwards was higher in the 30-d than the 7-d treatment for both the middle (Fig. 4A; 2-way ANOVA, day $F_{1,16} = 8.25$, $p = 0.01$) and base

layers (Fig. 4B; 2-way ANOVA, day $F_{1,16} = 19.48$, $p = 0.0004$). Furthermore, zebra mussel size influenced whether or not movement occurred from the middle (Fig. 4A; 2-way ANOVA, size $F_{3,16} = 45.04$, $p = 0.0001$) and base layer (Fig. 4B; 2-way ANOVA, size $F_{3,16} = 157.77$, $p = 0.0001$), where the smaller size classes exhibited greater upward migration than larger size classes. We did not find a significant interaction between size class and incubation time for the middle layer (2-way ANOVA, $F_{3,16} = 1.03$, $p = 0.41$). However, a significant interaction did occur in the base layer ($F_{3,16} = 3.63$, $p = 0.036$) as more smaller mussels migrated after 30 d.

Size class I (≤ 5 mm) mussels migrated upward significantly more often than size class II (6–13 mm) mussels or size class III (14–20 mm) mussels in both the middle and base vertical layers for both incubation periods (Fig. 4A, B; Tukey's MCT, $p < 0.05$). Size class IV (> 20 mm) mussels failed to move from either layer. Thus, more small mussels (< 13 mm) in the middle layer moved upward to the surface layer after 7 and 30 d (53% and 67%, respectively), than mussels that were > 20 mm (0% migration on both dates). The same trend occurred in the base layer where small mussels moved upwards after 7 and 30 d (54% and 69%, respectively), whereas larger mussels did not migrate.

In situ field survey.—We found significant differences in the frequency distribution of different sizes of zebra mussels among vertical strata in Lake Michigan colonies (Fig. 5; χ^2 test, $\chi^2_{3,0.05} = 14.38$, $p = 0.002$). Equal distributions of size classes did not occur in either the top ($\chi^2_{3,0.05} = 41.52$, $p = 0.0001$) or bottom ($\chi^2_{3,0.05} = 25.76$, $p = 0.001$) layers. Fewer size class IV mussels occurred in the surface layer (3%) than in the base layer (10%) (Fig. 5). Size class II mussels constituted $> 50\%$ of the mussels in both layers overall (Fig. 5). Dead mussels constituted 0.6% of the total in the surface layer, compared to 3% of the total in the bottom layer.

Discussion

Vertical gradients in 3-dimensional colonies

Vertical chemical gradients can develop in the interstitial water of densely organized assemblages of sessile aquatic organisms, especially at low current velocities. Many studies (Burkholder et al. 1990, Liehr et al. 1990, Blenkinsopp et al.



Vertical layer within zebra mussel colonies

FIG. 3. Percent mortality (+1 SE) of 4 zebra mussel size classes after 30 d in a 6-cm thick colony, subdivided into 3 vertical layers. Different small letters above bars represent statistical differences (Tukey's multiple comparison test, $p < 0.05$) between the vertical layers.

1991, Stevenson and Glover 1993, Johnson et al. 1997) have demonstrated this phenomenon occurring within algal biofilms. Although zebra mussel colonies are frequently 3-dimensional in structure, little is known about the quality of interstitial water and the zebra mussels' potential strategies for survival at the base of the colony. Our laboratory experiments verified the development of strong chemical gradients within zebra mussel colonies that occurred at densities similar to those reported by MacIsaac et al. (1991) in Lake Erie. Dissolved oxygen was significantly lower and $\text{NO}_3\text{-N}$ significantly higher in the base versus the surface of the laboratory colonies.

Influence of flow on interstitial water-quality conditions

Gradients measured in natural zebra mussel colonies in Lake Michigan were less developed than gradients in our laboratory experiment. This result was perhaps attributable to the lower densities of the colonies in Lake Michigan, and to wave action in nearshore Lake Michigan habitats, which continually refreshes interstitial water. Flow in our laboratory experiments was constant, horizontal, and unidirectional, but slow (1 cm/s), whereas local velocities near the Port of Indiana breakwall could exceed 100 cm/s, and current direction can be chaotic. Water in the laboratory flumes flowed horizontally through the isolated colonies, whereas colonies on the

breakwall are contiguous, and wave action tends to be propagated perpendicular to the breakwall. It is, therefore, difficult to estimate and compare the effect of water movement on water quality under the 2 differing conditions. Strong interstitial chemical gradients may be eliminated within dense colonies in ecosystems with significant flow, thereby allowing thicker colonies to form with minimal physiological stress. However, zebra mussels continue to spread into backwater areas and small inland lakes that do not feature strong flows (Horvath et al. 1996). Our results suggest that gradients of interstitial water quality may influence the distribution and survival within these populations with relatively low densities.

Interstitial chemical gradients within our laboratory colonies occurred for $\text{NO}_3\text{-N}$ and DO, but not $\text{NH}_4\text{-N}$. Ammonia is a common waste product generated by aquatic invertebrates, but it is chemically unstable in oxygenated environments, rapidly oxidizing to $\text{NO}_3\text{-N}$ via nitrification (Wetzel 2001). The high oxygen conditions in our experiment (lowest level = 6.0 mg/L), and presumably within Lake Michigan, produced an oxidizing chemical environment that favors $\text{NO}_3\text{-N}$ over the reduced form of dissolved inorganic N, $\text{NH}_4\text{-N}$. Toxic levels of $\text{NH}_4\text{-N}$ (3 mg/L; Nichols 1993) did not occur in our study because $\text{NH}_4\text{-N}$ was quickly converted to $\text{NO}_3\text{-N}$. Therefore, we used $\text{NO}_3\text{-N}$ levels as our indicator of waste accumulation and potential stress (James et al. 1997). We suggest that the

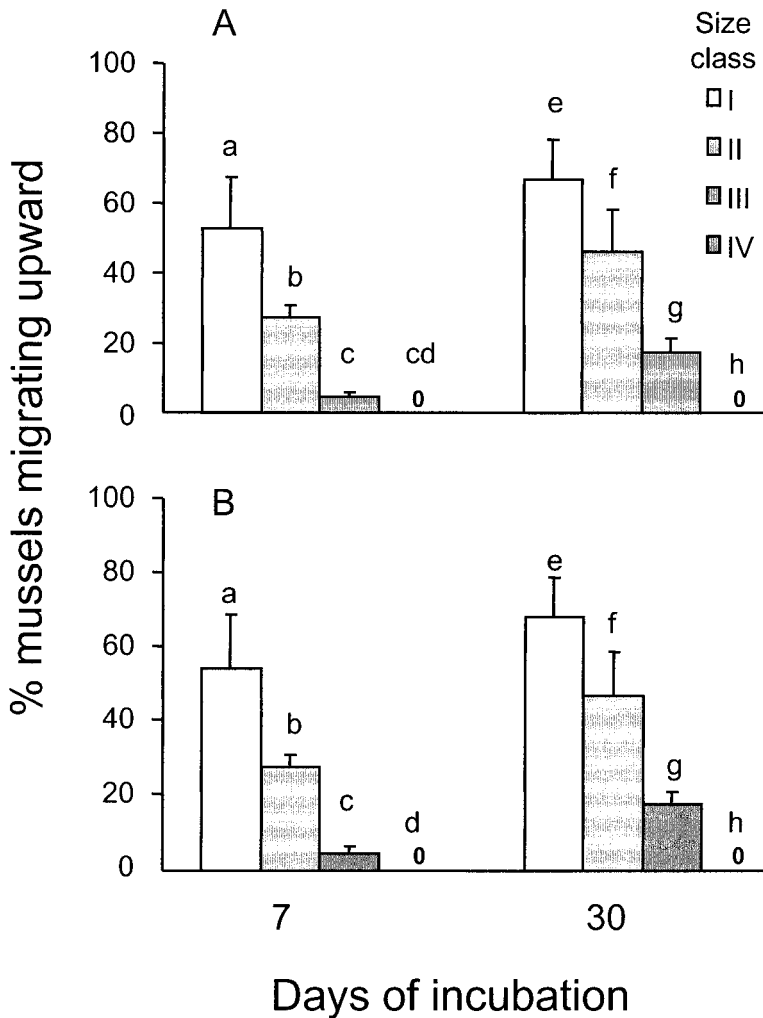


FIG. 4. Percent (+1 SE) of zebra mussels that moved upward after 7 and 30 d of incubation in a 6-cm thick colony under low flow. Data represent % of each size class that moved up from the middle layer (A) or base layer (B) of the colony. Different small letters above bars represent statistical differences (Tukey's multiple comparison test, $p < 0.05$) between size classes (both layers pooled) incubated the same amount of time (7 or 30 d).

most stressful conditions within the colonies occurred in the bottom layer where maximum $\text{NO}_3\text{-N}$ and minimum DO concentrations occurred. After just 4 h, $\text{NO}_3\text{-N}$ concentrations of our artificially constructed colonies reached a maximum of 0.835, 0.725, and 0.470 mg/L in the base, middle, and surface layers of the colony, respectively; minimum final $\text{NO}_3\text{-N}$ concentrations in the surface, middle, and base layers were 0.330, 0.420, and 0.450 mg/L, respectively. We presume that this level of $\text{NO}_3\text{-N}$ was detrimental to zebra mussel survival; however,

more experiments are needed to determine acute levels of $\text{NO}_3\text{-N}$ toxicity and how nitrate accumulation in colonies impacts zebra mussel survival, physiology, and reproduction over longer periods of time.

Behavioral strategies to avoid adverse conditions in dense colonies

A combination of stressors in addition to high $\text{NO}_3\text{-N}$ levels and low DO availability may have contributed to the movement of zebra mussels

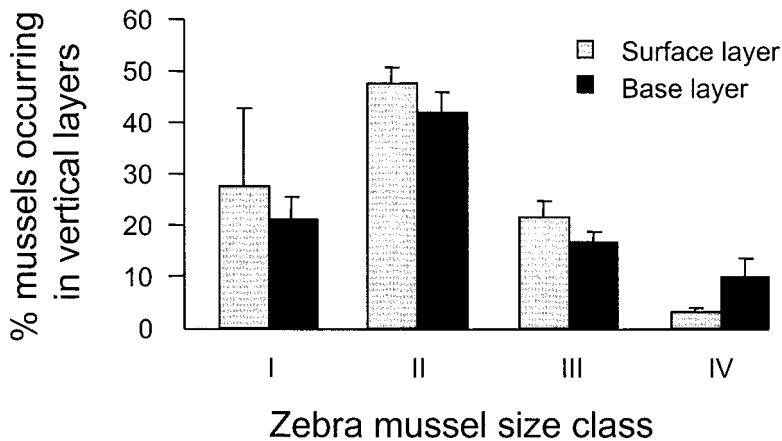


FIG. 5. In situ % vertical distributions (+1 SE) of 4 size classes of zebra in natural colonies in Lake Michigan ($n = 8$).

away from the base of the colonies in our laboratory experiments. Insufficient food resources at the base of the colony may also induce zebra mussel movement. The water in our flume was unfiltered (i.e., contained phytoplankton) and continually flowed slowly over the colonies. Interstitial water-quality gradients are likely responsible for mortality and movement of zebra mussels, but more experiments examining how individuals secure food resources among dense aggregates are needed to further speculate on the influence of food availability on survival of individuals living in groups.

Zebra mussels apparently colonize on top of one another as a mechanism to maximize population density in an environment limited by suitable colonization surface (Toczyłowski and Hunter 1997). However, the population benefit of increased density may be accompanied by substantial costs to individual zebra mussels. Thick (up to 8 mm) benthic algal mats represent a comparable structure to dense 3-dimensional zebra mussel colonies. For benthic algae, the benefits of living within a 3-dimensional assemblage include internal cycling of nutrients (Mulholland 1996) and an escape from grazers that cannot dislodge closely adhering basal cells from the substrate (Tuchman and Stevenson 1991). The cost of living at the base of a thick biofilm is resource limitation (Tuchman 1996) as a vertical gradient develops in resources such as light (Dodds 1992) and inorganic nutrients (Burkholder et al. 1990, Stevenson and Glover 1993). For zebra mussels, costs associated with colonial liv-

ing include vertical gradients in water chemistry as demonstrated in our study and reduced food availability near the base of the aggregate (NCT, unpublished data). The decrease in water quality at the base of thick zebra mussel colonies also has implications for other, native invertebrate species. Zebra mussels are able to colonize soft sediments (Berkman et al. 1998). As colonies of mussels expand onto soft substrates, the reduction in water quality created underneath the colony could be detrimental to burrowing mayflies (*Hexagenia* spp.), burrowing amphipods (*Diporeia* spp.), and other infaunal species.

Size-dependent mortality and migration in zebra mussels

We explored the mechanisms for survival that zebra mussels might use when they occur in dense colonies under stressful conditions, and demonstrated the propensity of small individuals to move upward, away from the base of the colonies and toward the surface. We found a 15% reduction in DO and an 88% increase in $\text{NO}_3\text{-N}$ at the base of our laboratory colonies after only 4 h. These adverse conditions will increase over time, as likely occurred in our 7- and 30-d mortality and migration experiments. Significantly higher mortality occurred at the base versus the middle or surface vertical layers of zebra mussel colonies after 30 d, which indicated that movement is advantageous for survival.

Small individuals (<13 mm) were more likely to escape from the base of the colonies and ex-

hibited much more mobility than large mussels (>20 mm). This difference may occur because large mussels can act as an attachment surface for other mussels (Cawein 1993) and can become the nucleus of a cluster, making it impossible for the large central mussels to maneuver. Matthews and McMahon (1999) found that, in response to extreme hypoxia, smaller mussels had a lower tolerance to low DO conditions than larger mussels, which may partially explain the tendency of smaller individuals to escape. However, this escape strategy from adverse interstitial water conditions goes against the natural tendency of zebra mussels to avoid light (Zhang et al. 1998, Marsden and Lanksy 2000). Although large individuals did not migrate out of the middle or base of the colonies, their exposure to the more stressful conditions (e.g., lower DO, higher NO₃-N, potentially lower food availability) within the base layer produced significantly higher mortality within the base than smaller individuals. After 30 d, our laboratory colonies showed trends in size-class distribution similar to natural colonies in Lake Michigan. We suggest that movement by small individuals may be partially responsible for this pattern. However, veligers also tend to settle out of the water onto the surface of colonies, so that older, larger individuals are at the base and the smaller, newer colonists are at the top as a colony forms. Additional field experiments that investigate the % of migrants versus new colonizers are needed to fully understand natural field distributions of zebra mussels.

Previous studies regarding the environmental tolerances of zebra mussels to hypoxia and other stresses have focused on individuals or small aggregates (Aldridge et al. 1995, Clarke and McMahon 1996, Matthews and McMahon 1999) with extrapolation to the colony scale. However, based on our experiments, we suggest that the degree of environmental stress an individual experiences strongly depends upon its spatial position within the colony, whereas its ability to move to a more advantageous position appears to be a function of size.

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