



Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan

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ABSTRACT

Trends in density, biomass, population structure, and nutritional state of *Dreissena polymorpha* and *Dreissena rostriformis bugensis* were examined in southern Lake Michigan between the 1990s and 2008. Density and biomass of *D. polymorpha* increased to a peak in the early 2000s and then declined. In contrast, *D. rostriformis bugensis* was first found in the southern basin in 2001 and has continued to increase in density or biomass at all depths ever since. In 2008, maximum mean density of *D. rostriformis bugensis* occurred at 16–30 m (19,000/m²), but maximum biomass (AFDW) occurred at 31–50 m (43.9 g/m²). *D. rostriformis bugensis* has only recently (since 2005) began to increase at depths >50 m. When both species were present in 2004 at depths <50 m, a condition index (CI) for *D. rostriformis bugensis* was 27% higher, and shell weight per shell length was 48% lower compared to *D. polymorpha*. For *D. rostriformis bugensis*, CI decreased in 2008 compared to 2004 at 25 m and 45 m, but biochemical content (lipid, glycogen) did not. Seasonal changes in both RNA/DNA ratio (growth) and ETS (metabolic activity) in *D. rostriformis bugensis* were unaffected by reproductive activity, and only ETS appeared to change seasonally relative to bottom temperatures. Spawning of *D. rostriformis bugensis* occurred in late summer at 25 m, but occurred in spring at 45 m. Veliger densities peaked in both spring and late summer at both depths. Future population expansion (biomass) is expected to be most rapid at depths >50 m.

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Introduction

In the late 1980s, two invading bivalve mollusks, *Dreissena polymorpha* (zebra mussel) and *Dreissena rostriformis bugensis* (quagga mussel), became established in the Great Lakes and have since spread into a wide range of habitats in all five lakes. *D. polymorpha* expanded rapidly and reached greatest abundances in shallow, nearshore regions and bays; *D. rostriformis bugensis* expanded more systematically but over time has become abundant in both nearshore and offshore regions (Mills et al., 1999; Patterson et al., 2005; Nalepa et al., 2009). The capacity of both species to filter large volumes of water, assimilate suspended material, and excrete nutrients has caused broad scale and fundamental changes in the cycling of energy and nutrients through the Great Lakes system (Nalepa and Fahnenstiel, 1995; Vanderploeg et al., 2002; Hecky et al., 2004).

For various reasons, less is known of the population dynamics, life habits, and nutritional state of *D. rostriformis bugensis* compared to *D. polymorpha*, particularly in deep, consistently cold regions. For one, with the exception of eastern Lake Erie, the spread of *D. rostriformis*

bugensis into offshore regions of the Great Lakes is generally recent (Watkins et al., 2007; Nalepa et al., 2009). In Europe, this species is confined to relatively shallow regions (Mills et al., 1996). Unique to the Great Lakes is the presence of two phenotypes of *D. rostriformis bugensis*, the hypolimnetic phenotype (“profunda”) and the epilimnetic phenotype, which complicates our understanding of spatial distributions and life cycles. These phenotypes are genetically similar but have different environmental preferences (Spidle et al., 1994; Claxton and Mackie, 1998).

A number of studies have compared *D. rostriformis bugensis* with *D. polymorpha*, and results indicate that *D. rostriformis bugensis* has a higher assimilation efficiency (Baldwin et al., 2002), a lower respiration rate (Stoeckmann, 2003), and can spawn at colder temperatures (Roe and MacIsaac, 1997; Claxton and Mackie, 1998). Also, *D. rostriformis bugensis* has higher filtration rates during the warmer months (Diggins, 2001). These attributes theoretically allow it to colonize deep offshore regions where food availability is limited and temperatures are constantly cold, and to outcompete *D. polymorpha* in shallow, warm regions where food availability has been reduced by dreissenid-filtering activities (Wilson et al., 2006).

In this paper, we document changes in population density and biomass of *D. rostriformis bugensis* and *D. polymorpha* in southern Lake Michigan between 1992 and 2008. Based on long-term sampling, we knew that densities of *D. polymorpha* were declining and those of *D.*

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rostriformis bugensis were increasing in this portion of Lake Michigan since the early 2000s (Nalepa et al., 2009). So besides documenting recent population shifts, we also compared the nutritional state (relative tissue mass and biochemical content) of the two species in nearshore and offshore habitats. Our goals were to better understand the relationship between nutritional state and dreissenid population trends as *D. polymorpha* was displaced by *D. rostriformis bugensis*, and also to perhaps better predict the potential for future population growth of *D. rostriformis bugensis* in the offshore region. Further, we examined seasonal spawning patterns, recruitment, and metabolic activity (RNA/DNA and ETS) of *D. rostriformis bugensis* at sites above (25 m) and below the thermocline (45 m).

Methods

Population trends in *D. polymorpha* and *D. rostriformis bugensis* in the southern basin of Lake Michigan were derived from surveys at 40 sites that were part of a long-term benthic monitoring program (Fig. 1; Nalepa 1989; Nalepa et al., 1998). *D. polymorpha* was first reported in southern Lake Michigan in 1989 (Marsden et al., 1993), and our initial post-*Dreissena* sampling at the 40 sites occurred in 1992–1993. Thereafter, we sampled every year between 1998 and 2008. Sites were sampled in spring, summer, and fall in 1992, 1993,

1998, 1999, 2004, and 2005; otherwise, samples were collected in late summer/fall.

Samples at each site were taken in triplicate with a Ponar grab (sampling area = 0.046 m²). Sediments were washed through an elutriation device fitted with a 0.5-mm mesh screen, and retained residue preserved in 5% formalin containing rose bengal stain. In the laboratory, all dreissenids were picked and counted under a low-power magnifier lamp (1.5×). Samples with extremely high numbers were subsampled by selecting a random quadrat of individuals that were evenly distributed in a gridded tray. For purposes of analysis, the 40 sites were placed into four depth intervals: 16–30 m ($n = 11$), 31–50 m ($n = 12$), 51–90 m ($n = 11$), and >90 m ($n = 6$). These depth intervals represent 22.6%, 11.6%, 32.2%, and 33.6% of the total area of the southern basin.

Dreissenids were collected for assessment of nutritional state at sites along two depth transects in 2004 and 2008. Two sites were located off Waukegan, IL, and two sites were located off Muskegon, MI. The Waukegan sites were included in the long-term sampling program and were at 20 m (H-8; 42°23.92'N, 87°46.25'W) and 45 m (B-7; 42°22.00'N, 87°40.00'W), whereas the Muskegon sites were at 25 m (M-25; 43°12.00'N, 86°22.67'W) and 45 m (M-45; 43°11.43'N, 86°25.72'W) (Fig. 1). These sites were considered representative of the west and east sides of the basin, respectively. Both species were

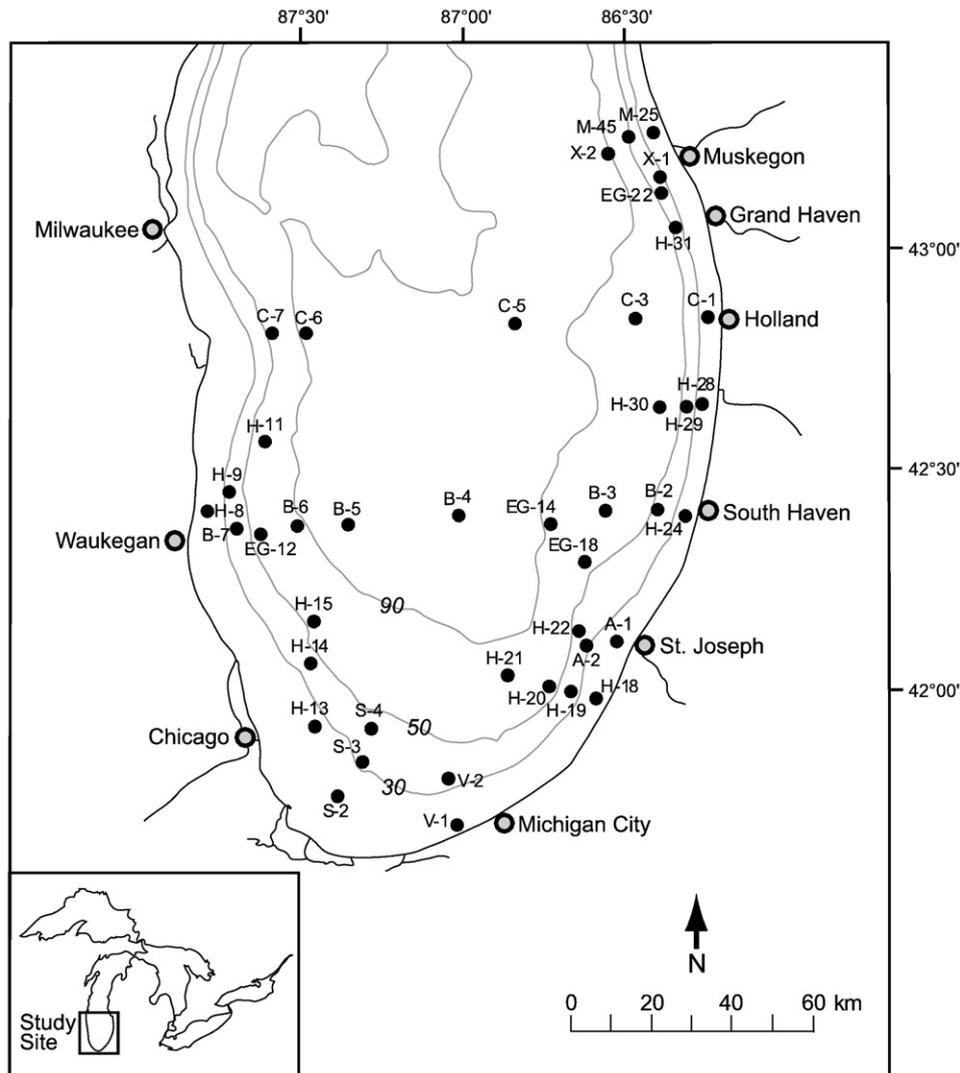


Fig. 1. Location of sampling sites in southern Lake Michigan. All sites except M-25 and M-45 were sampled as part of a monitoring program to assess long term changes in the benthic community. *Dreissena* for physiological condition and biochemical content were collected at H-8 and B-7 in 2004, and at M-25 and M-45 in 2004 and 2008. Depth contours are at 30 m, 50 m, and 90 m.

collected at all four sites in 2004, but only *D. rostriformis bugensis* was collected in 2008 and only at the two Muskegon sites. By 2008, *D. polymorpha* was very rare. In 2004, the Waukegan sites were sampled on four dates (May 27, Jul 1, Aug 25, and Sep 29), and the Muskegon sites were sampled on seven dates (Apr 20, Jun 3, Jun 23, Jul 21, Aug 31, Sep 21, and Oct 27). In 2008, the Muskegon sites were sampled on eight dates (Apr 8, Apr 30, Jun 4, Jul 7, Aug 12, Sep 2, Sep 29, and Nov 3). On each sampling date regardless of year, dreissenids were collected with either a Ponar grab or a benthic sled, gently rinsed in lake water, placed in coolers, and kept at 4 °C during transport back to the laboratory. In addition to collecting live animals, triplicate Ponar grabs and duplicate vertical net tows were taken on each date in 2008 at Stations M-25, M-45, and X-2 (93 m; 43°12.00'N, 86°31.00'W). The grab samples were washed through a 0.5-mm mesh net, and retained mussels were preserved and later counted as given above. It was initially planned to collect live mussels at X-2 for nutritional state, but too few mussels of various sizes were present.

The vertical net tows were taken to obtain estimates of dreissenid veligers. A conical plankton net (64- μ m mesh openings, 1.5 m long, 0.3 m diameter) was hauled vertically from 1 m off the bottom to the surface; actual distance of the net haul was determined with a block/cable metering system. Net contents were preserved in 5% sugared formalin. In the laboratory, a subsample of a known sample volume was taken with a Hensen–Stempel pipette so that at least 100 veligers or 10% of the sample were counted per tow. On each sampling date in 2008, bottom water temperatures (1 m above bottom) were measured with a SeaBird CTD. Measurements were taken at 15 m and 45 m (M-45) except on Sept 29 when temperatures were taken at 25 m (M-25) instead of at 15 m. For purposes of this study, it was assumed that bottom temperatures at 15 m and 25 m were similar. These depths are above the thermocline in this region of Lake Michigan, and the water column is horizontally well mixed; bottom temperatures undergo broad fluctuations because of seiches, longshore currents, and upwellings/downwellings (Mozley and Winnell, 1975).

Within 48 hours after collection, live mussels were separated into five size categories based on relative shell length (9–12 mm, 13–15 mm, 16–18 mm, 19–21 mm, and 22–25 mm). Five individuals in each size category were cleaned of all shell debris, blotted dry, and then weighed to obtain whole, wet weight. After removal from the shell, soft tissue was placed into a combusted, preweighed aluminum planchet, dried at 60 °C for at least 48 h, and weighed to determine shell-free dry weight (DW). Tissues were then ashed at 550 °C for 1 h and weighed again for determination of ash-free-dry-weight (AFDW). The corresponding shell was measured (length; SL), dried at room temperature for at least 48 h, and weighed.

These measurements of soft tissue and shell were used to determine the length–weight relationship and the condition index (CI). The former is defined by the general allometric relationship $AFDW = aSL^b$ and presented as $\log_e AFDW \text{ (mg)} = b + a \log_e SL \text{ (mm)}$. The relationship has been frequently used as an index of nutritional state in dreissenids, with the weight of a standard 15-mm mussel providing a convenient way to compare the state of individuals in different populations (Nalepa et al., 1993, 1995; Chase and Bailey, 1999a). The condition index (CI) is often used to assess the nutritional state of marine bivalves (Austin et al., 1993; Rheault and Rice, 1996; Mercado-Silva, 2005) and is calculated as follows:

$$CI = \frac{\text{dry soft tissue wt (g)} \times 1000}{\text{internal shell capacity (g)}}$$

The internal shell capacity is determined by subtracting dry shell weight from whole wet weight (soft tissue and shell) of a cleaned animal (Crosby and Gale, 1990). In essence, this index assesses the proportion of available internal shell cavity that is utilized by soft tissue and is useful in comparing bivalve species with different shell dimensions (width vs. length), as is the case for *D. rostriformis bugensis* and *D. polymorpha* (Dermott and Munawar, 1993). Since whole wet

weight includes unassociated water within the shell cavity, some error is introduced when the shell opens and this free water is lost. However, we assumed that the number of these “gapped” individuals is small and random between the two species.

In addition to determining AFDW/SL and CI, total lipid and glycogen content were measured in both species in 2004 and in *D. rostriformis bugensis* in 2008. ETS (electron transport system), RNA/DNA (ribonucleic acid/deoxyribonucleic acid), and reproductive status were measured in *D. rostriformis bugensis* only in 2008. In 2004, soft tissue from 3 to 10 individuals in each of the three largest size categories of each species were placed into cryogenic vials and kept frozen at –80 °C until analysis. In 2008, four individuals in each of the three largest size categories were separately placed into vials for each analysis ($n = 12$ for each variable), and vials were stored at –80 °C.

Lipid content was determined by chloroform:methanol extraction followed by subsequent analysis using the microcolorimetric SPV approach (sulfophosphovanillin). Tissue samples were taken from the freezer and freeze-dried. Once dry, the tissue was pulverized, and a 2- to 5-mg subsample (measured to nearest 0.001 mg) was placed into a glass test tube (50 mm \times 6 mm) followed by the addition of 200 μ L of chloroform:methanol (2:1). Samples were homogenized for 30 s at 5000 rpm using a variable-speed rotary tool (Dremel model no. 380-5) with a glass bead pestle while immersed in ice to minimize evaporation. This homogenate was decanted into a 1.5-mL conical centrifuge tube. Another 200 μ L of the 2:1 chloroform:methanol extraction solvent was added to the centrifuge tube as a rinse, homogenized briefly (~5 s), and decanted to a total extract volume of 400 μ L (Gardner et al., 1985). To purify the lipid extract, 200 μ L of 0.9% NaCl solution was added to each sample, which was then mixed by vortexing (Folch et al., 1956). Centrifuge tubes were then placed in an Eppendorf centrifuge for 15 s at 8000 rpm, or a relative centrifugal force (G-force) of 5220. At this G-force, the phase separation occurs almost immediately, and increasing time did not improve separation in controlled trials. This phase separation results in nonlipid compounds and methanol being partitioned into the upper aqueous phase and the lipid–chloroform extract in the lower organic phase. From this organic lower phase, a microsampling pipette was used to remove a 100- μ L aliquot for microcolorimetric SPV analysis.

Microcolorimetric SPV analysis followed the methods of Van Handle (1985). Culture tubes (13 mm \times 100 mm) containing 100 μ L of lipid extract were heated at 100 °C for 15 min on a dry bath heater to remove the chloroform solvent. We made certain to get complete evaporation of the chloroform as it has a positive interference with the phosphovanillin reagent. Then, 0.25 mL of 96% H₂SO₄ was added to the bottom of the culture tube to make complete contact with the lipid residue, and samples were then vortexed and heated for 10 min at 100 °C on a dry bath heater. Five milliliters of the colorimetric SPV reagent (3 g vanillin, 0.5 L deionized water, 2 L concentrated phosphoric acid, stored in the dark) was added in each tube and thoroughly mixed by vortexing. After sitting at room temperature for 30 min, the absorbance (A) was measured, using a Turner spectrophotometer. Solvent blanks were run through the entire procedure and used to “zero” the spectrophotometer. We used pure cod liver oil dissolved in 2:1 chloroform:methanol solvent mixture (10 mg/mL) as a stock standard. Working volumes of 0 (blank), 10, 20, 30, 40, and 50 μ L of this standard solution were used to develop a standard curve for lipids (absorbance (A, nm) vs. mass of the standard (mg)), with the percent dry mass of lipids (PL) calculated as follows:

$$PL = \left(A * V_p * 100 \right) / \left(V_s * S_s * M_f \right),$$

where V_p , V_s , and M_f are as described earlier ($V_s = 100 \mu$ L in this study), A (nm) is the absorbance at 525 nm, S_s is the slope of the standard's regression line (nm/mg), M_f is the measured mass of the dry mussel sample (mg), and V_p is the volume ratio between extract fraction used for colorimetric SPV reactions and the total solvent

mixture used for extraction. Lipid content was reported on a percent dry weight basis.

After freeze-drying, whole tissue samples for glycogen analysis were weighed so that the volume of glycogen buffer (phosphate) could be predetermined to keep the homogenate within the range of 1–2 mg/mL. Methods followed those of Roehrig and Allred (1974) for the reduction of glycogen to glucose, and Sigma Kit 510 was used for glucose analysis. Tissues were homogenized in 0.05 M phosphate buffer (pH 4.8–5.0) in a 30-mL Teflon/glass tissue grinder over ice for 30 s, which was then followed by heat exposure in boiling water for 90 s. A 3-mL aliquot of homogenate was transferred into ultra centrifuge tubes and centrifuged at 10,000 ×g for 20 min at 0 °C. A 0.5-mL aliquot was transferred from the centrifuge tube into a 13 × 100-mm test tube and combined with 0.5 mL of amyloglucosidase. The mixture was vortexed lightly, and then incubated in a water bath at 37 °C for 2 h. Also incubated were amyloglucosidase blanks (no glycogen) and glycogen standards made from *Mytilus*. After incubation, 0.5 mL was transferred to another test tube and incubated with 2.5 mL of glucose oxidase/peroxidase (GO/PO) enzymes at 37 °C for 30 min. GO/PO blanks (0.5 mL buffer and 2.5 mL GO/PO) and glucose standards with GO/PO were also incubated. All absorbances were read with a Turner spectrophotometer and reported on a percent dry weight basis.

Tissues samples for ETS analysis were lightly blotted and wet weights determined before being frozen and stored at –80 °C. Methods for ETS analysis followed those of Bämstedt (1980, 2000). At the time of analysis, tissue samples were thawed and the entire sample homogenized in 0.1 M phosphate buffer. The volume of the buffer was varied to keep a homogenate concentration of 2–5 mg/mL. Three milliliters of the homogenate was transferred to a centrifuge tube and clarified at 10,000 ×g for 10 min in a refrigerated centrifuge at 0 °C. One milliliter of the resulting supernatant was pipetted into each of two 16 × 100-mm culture tubes, with one of the tubes receiving 1 mL iodinitrotetrazolium reagent, and the other receiving phosphate buffer to serve as a turbidity blank. These tubes were then incubated at 20 °C. After 20 min, the reaction was stopped with 1-mL quench solution, and absorbances measured at 490 nm using a Turner spectrophotometer. The turbidity blank was used to zero the spectrophotometer for each individual sample. ETS was determined according to the following equation:

$$\text{ETS} = \frac{(A/C) \times (60/I) \times ((V \times H) / (S \times M)) \times (32/2)}{\text{electron transport system (activity measured as } \mu\text{gO}_2 \text{ g/wet wt/h)}}$$

A corrected sample absorbance (cm⁻¹)
 C constant molar absorbance for INT-formazan determined by standard curve (μmol/mL/cm)
 I incubation time (minutes)
 V final reaction volume (mL)
 H total homogenate volume (mL)
 S incubated volume (mL)

Tissue samples for nucleic acid analysis were extracted immediately upon removal from the –80 °C freezer. The extraction was modified from established protocols for measuring nucleic acids (Gorokhova and Kyle, 2002; Hook et al., 2008) to allow the inclusion of the whole mussel, which ranged in wet weight between 30 and 300 mg. Tissue samples were placed in 5 mL extraction buffer (1% N-lauroyl sarcosine sodium salt in TE buffer) and homogenized over ice using a Teflon pestle and glass tube grinder. To achieve working concentrations similar to previous studies (Gorokhova and Kyle, 2002; Hook et al., 2008), a 100-μL subsample of the raw extract was combined with 1400 μL of extraction buffer in a conical snap cap centrifuge tube. After homogenization, samples were subjected to three repeated sequences of ultrasonic (30 s) and ice (1 min) baths. Samples were then put on a shaker table at 170 rpm at room temperature for 2 h. Each homogenate was assayed in triplicate using

a microplate fluorescence reader (FLx800 Biotek Instruments, filters: 485 nm for excitation and 528 nm for emission) and 96-well black flat bottom plates. Plates were scanned with 1 s well measurement time and 10 measurements per well. Plates were also loaded in duplicate with RNA standards ranging from 0.16 to 2.57 μg/mL, DNA standards ranging from 0.04 to 0.68 μg/mL, blanks, and negative controls. The fluorescence of each well was first quantified after RiboGreen addition (70 μL/well; 5 min incubation at room temperature) and then for a second time after RNase addition (5 μL/well; 30 min incubation at 37 °C). The first fluorescence gave a measure of total nucleic acids, while the second gave a measure of DNA since RNA in each well was digested by RNase. The second measure was then subtracted from the first to obtain only DNA fluorescence. Each sample's fluorescence was then compared to the standard curve to quantify the amount of RNA and DNA in each subsample.

Reproductive status of *D. rostriformis bugensis* was determined at 25 m (M-25) and 45 m (M-45) on each date in 2008. Within 48 h of collection, the gonads of 10 female mussels of at least 20 mm in shell length (range 20–26 mm) were examined under 100× magnification using a squash mount preparation. Development stage of the oocytes was categorized as given in Nichols (1993): Stage 1, no nuclei or germinal vesicle; Stage 2, visible nuclei but no germinal vesicle; Stage 3, <50% of oocytes have nuclei and germinal vesicle; Stage 4, >50% of oocytes have nuclei and germinal vesicle; Stage 5, only a few Stage 4 oocytes remain (spawning has occurred). The reproductive status was reported as the percentage of individuals at each stage.

Population biomass was estimated separately for *D. polymorpha* and *D. rostriformis bugensis* from size frequencies and derived length–weight relationships. Shell lengths of mussels were measured at 18 of the 40 monitoring sites in each year between 1998 and 2008 (late summer/fall only). These sites were considered representative of the southern basin and chosen based on depth and location: C-1, H-8, H-13, and H-24 in the 16- to 30-m interval; B-2, B-7, H-14, H-19, and H-31 in the 31- to 50-m interval; B-3, B-6, H-15, H-20, and H-21 in the 51- to 90-m interval; and B-4, B-5, and X-2 in the >90-m interval. Mussels in each replicate sample were measured and then binned into 1-mm size categories from 1 to 33 mm. When the number of mussels in an individual sample was large, it was subsampled as described above. The minimum number of mussels measured in each subsample was 100. To determine total biomass (as AFDW), the number of individuals in each size category was multiplied by the AFDW of an individual within that category (midshell length), and all category weights were summed. Weight per midshell length in each size category was calculated from the length–weight regressions determined in 2004 and 2008. Two regressions were used for *D. polymorpha*, one for sites in the 16- to 30-m interval, and the other for sites in the three deeper intervals. These two regressions represented relationships above and below the thermocline, respectively. The former regression was derived from mussels collected at Stations H-8 and M-25 on all dates in 2004, and the latter regression was derived from mussels collected at Stations B-7 and M-45, also on all dates in 2004. The regression used for *D. rostriformis bugensis* depended on depth interval as well as sampling year. For years between 1998 and 2004, depth-specific regressions were derived from mussels from the same sites as for *D. polymorpha* on all sampling dates in 2004. For years between 2005 and 2008, the regression used was derived from mussels collected on all dates in 2008. As given, mean biomass at each interval is based on estimates from all 40 sites. Biomass at sites where mussels were not measured ($n=22$) was estimated by first determining the mean proportion of the population within each size category by depth interval and year for the measured sites, multiplying the proportion by total density at the nonmeasured sites, and then determining category-specific weights as above.

Yearly changes in size frequencies over the period 1998–2008 for both *D. polymorpha* and *D. rostriformis bugensis* were examined by binning individuals into 5-mm size categories (0–5 mm, 5–10 mm,

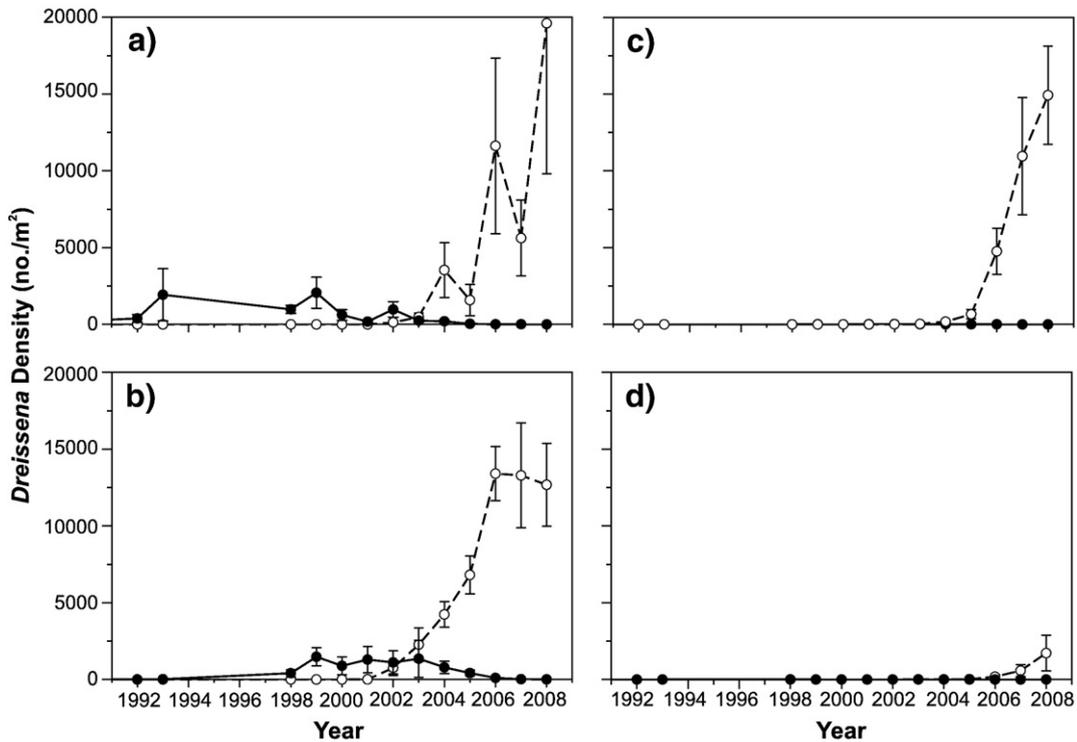


Fig. 2. Mean (\pm SE) density (no./m²) of *Dreissena* at each of four depth intervals: (a) 16–30 m, (b) 31–50 m, (c) 51–90 m, and (d) >90 m in southern Lake Michigan, 1992–2008. No samples were collected in 1994–1997. *Dreissena polymorpha* = solid circle/solid line; *D. rostriformis bugensis* = open circle/dashed line.

10–15 mm, 15–20 mm, 20–25 mm, >25 mm), and then determining the mean percentage within each size category. Size frequencies were only determined for those years and depth intervals in which mean densities exceeded 100/m². Seasonal changes in size frequencies in

2008 were determined from mussels collected in the Ponar grab at M-25, M-45, and X-2.

Differences between depths (sites) and years for indicators of nutritional state were tested using ANOVA (two-way) or a paired

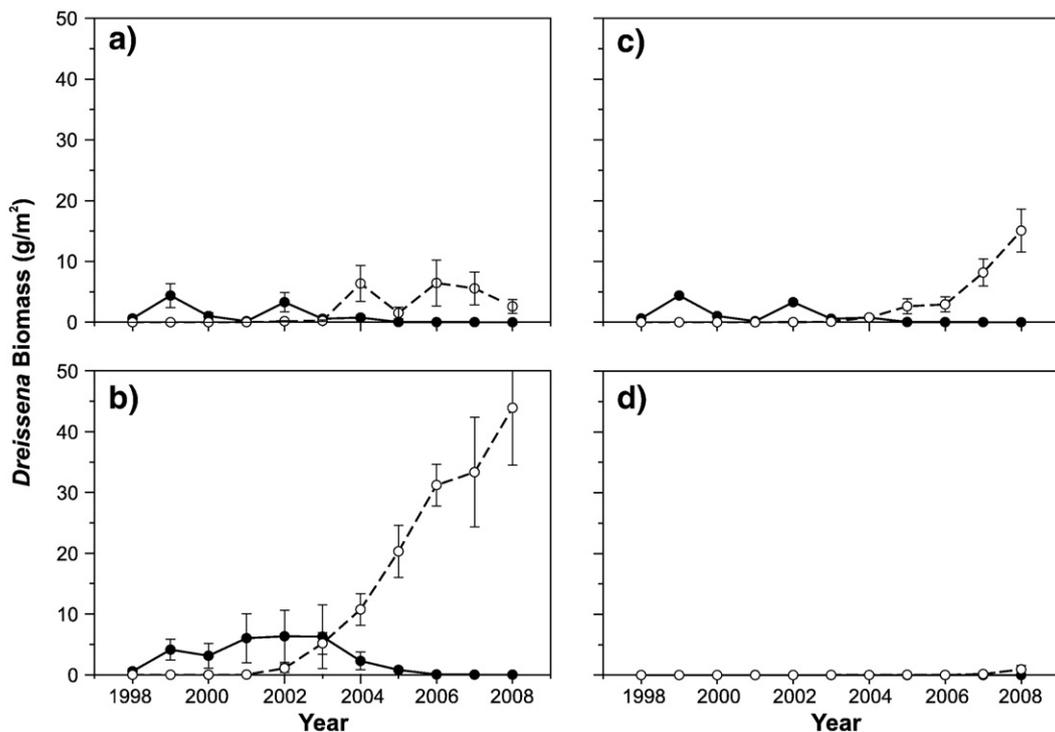


Fig. 3. Mean (\pm SE) AFDW biomass (g/m²) of *Dreissena* at each of four depth intervals: (a) 16–30 m, (b) 31–50 m, (c) 51–90 m, and (d) >90 m in southern Lake Michigan, 1998–2008. *Dreissena polymorpha* = solid circle/solid line; *D. rostriformis bugensis* = open circle/dashed line.

Table 1
Percentage of the population within various size categories at four depth intervals in 1998–2008. Size categories based on shell length. Also given is the mean size (mm) of an individual in the population. *Dreissena polymorpha* = (D.p.), *Dreissena rostriformis bugensis* = D.r.b.

Depth/year	Size Interval												Mean size	
	0–5 mm		5–10 mm		10–15 mm		15–20 mm		20–25 mm		>25 mm		D.p.	D.r.b.
	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.
16–30 m														
1998	87.8	–	6.6	–	3.9	–	0.9	–	0.7	–	0.1	–	3.1	–
1999	29.5	–	43.0	–	16.9	–	10.7	–	0.0	–	0.0	–	7.0	–
2000	48.2	–	30.0	–	12.9	–	9.0	–	0.0	–	0.0	–	6.1	–
2001	50.6	–	25.7	–	0.4	–	23.3	–	0.0	–	0.0	–	5.5	–
2002	14.4	–	29.6	–	31.1	–	15.4	–	5.1	–	4.3	–	10.6	–
2003	31.6	–	31.6	–	22.9	–	8.3	–	5.6	–	0.0	–	7.3	–
2004	27.9	44.8	16.7	23.0	40.2	27.4	14.8	4.0	0.5	0.7	0.0	0.1	9.1	7.0
2005	–	39.9	–	52.9	–	4.3	–	2.8	–	0.0	–	0.0	–	5.5
2006	–	80.0	–	11.6	–	5.4	–	2.9	–	0.0	–	0.0	–	3.6
2007	–	59.9	–	23.5	–	14.7	–	1.3	–	0.2	–	0.6	–	5.5
2008	–	90.5	–	6.6	–	2.1	–	0.5	–	0.3	–	0.0	–	3.1
31–50 m														
1998	69.8	–	25.2	–	3.3	–	1.4	–	0.3	–	0.0	–	5.0	–
1999	19.1	–	38.0	–	19.3	–	23.1	–	0.5	–	0.0	–	8.5	–
2000	31.0	–	31.8	–	27.4	–	7.4	–	1.9	–	0.4	–	8.8	–
2001	2.9	–	24.2	–	32.3	–	34.2	–	5.9	–	0.5	–	12.7	–
2002	17.5	53.7	10.9	39.4	33.9	2.0	18.7	1.4	18.8	3.6	0.2	0.0	12.7	5.5
2003	28.7	43.3	28.1	18.3	15.7	23.3	16.5	13.9	8.6	1.2	2.5	0.0	8.7	8.1
2004	36.0	44.1	35.1	29.4	21.9	17.6	6.6	6.9	0.2	1.6	0.2	0.3	7.6	7.2
2005	31.5	36.9	44.9	22.3	11.5	15.2	12.0	22.3	0.0	3.1	0.0	0.2	6.5	8.7
2006	–	53.0	–	20.5	–	10.1	–	8.2	–	7.8	–	0.4	–	6.9
2007	–	37.5	–	27.1	–	19.9	–	11.9	–	3.3	–	0.3	–	8.4
2008	–	33.4	–	22.2	–	19.6	–	18.3	–	5.7	–	0.8	–	9.7
51–90 m														
2006	–	87.4	–	10.2	–	1.2	–	0.3	–	0.6	–	0.3	–	3.6
2007	–	72.6	–	18.0	–	7.3	–	1.4	–	0.5	–	0.2	–	4.6
2008	–	65.0	–	14.1	–	18.5	–	2.1	–	0.2	–	0.1	–	5.1
>90 m														
2007	–	68.0	–	31.8	–	0.0	–	0.2	–	0.0	–	0.0	–	3.9
2008	–	83.6	–	11.5	–	3.3	–	0.2	–	0.1	–	0.4	–	3.9

t-test. All indicators reported as percentages were transformed using the arcsine transformation before analysis.

Results

Yearly trends in density, biomass, and size frequency

Trends in densities of *D. polymorpha* and *D. rostriformis bugensis* between 1992 and 2008 at the four depth intervals are given in Fig. 2. Over the entire period, *D. polymorpha* was mainly found at sites <50 m. Mean densities of this species at 16–30 m peaked at 2064/m² in 1999, varied between 183/m² and 971/m² in 2000–2004, and then declined to 0/m² by 2008. Temporal trends at 31–50 m were generally similar; mean densities peaked at 790–1477/m² between 1999 and 2003, and then declined to near 0/m² by 2008.

The decrease in densities of *D. polymorpha* after 2003 was temporally coincident with the expansion of *D. rostriformis bugensis* in the southern basin. *D. rostriformis bugensis* was first found in northern Lake Michigan in 1997 (Nalepa et al., 2001) but was not found in our study area until 2001. Initial densities increased at sites in the two shallowest intervals in 2001–2002, increased at sites in the 51- to 90-m interval in 2005, and increased at sites in the >90-m interval in 2007 (Fig. 2). As of 2008, mean densities were still increasing at all intervals except at 31–50 m. At this interval, mean densities were generally stable between 2006 and 2008, ranging between 12,600 and 13,400/m². Greater mean density at 16–30 m in 2008 compared to 2007 (19,614/m² vs. 5622/m²; Fig. 2) was mostly a result of a large number of small, newly settled individuals at just two sites on the east side of the lake. Densities at C-1 and H-24 in 2008 were 80,196/m² and 82,853/m², respectively, and >99% of individuals

Table 2
Mean (\pm SE) density (no./m²), condition index, lipid content (% dry weight), and glycogen content (% dry weight) of *Dreissena polymorpha* (D.p.) and *Dreissena rostriformis bugensis* (D.r.b.) at sites on the east (M-25, M-45) and west (H-8, B-7) of southern Lake Michigan in 2004 and 2008. Site depths: M-25 = 25 m, M-45 = 45 m, H-8 = 20 m, B-7 = 45 m.

Year/site	Density (no./m ²)		Condition index		Lipid (%)		Glycogen (%)	
	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.	D.p.	D.r.b.
2004								
M-25	64 \pm 45	963 \pm 291	70.8 \pm 2.3	82.2 \pm 1.9	10.4 \pm 1.0	14.3 \pm 0.9	2.9 \pm 0.4	2.7 \pm 0.3
M-45	942 \pm 511	8,748 \pm 1,740	38.9 \pm 1.0	60.6 \pm 1.0	10.0 \pm 0.3	11.1 \pm 0.5	1.6 \pm 0.2	2.5 \pm 0.2
H-8	350 \pm 250	674 \pm 558	41.6 \pm 1.4	60.8 \pm 2.2	8.4 \pm 0.3	8.3 \pm 0.5	1.0 \pm 0.2	1.3 \pm 0.2
B-7	67 \pm 44	533 \pm 148	63.2 \pm 1.8	89.7 \pm 1.9	15.0 \pm 0.9	16.2 \pm 1.3	3.8 \pm 0.5	3.1 \pm 0.3
2008								
M-25	0 \pm 0	4212 \pm 2,371		74.5 \pm 2.4		15.8 \pm 0.4		10.9 \pm 0.4
M-45	0 \pm 0	13,023 \pm 1347		41.6 \pm 1.1		12.1 \pm 0.4		4.7 \pm 0.3
H-8	0 \pm 0	1192 \pm 573						
B-7	0 \pm 0	9060 \pm 2533						

Table 3

Relationship between shell length (SL, mm) and tissue ash-free dry weight (AFDW, mg) for *Dreissena polymorpha* (*D.p.*) and *Dreissena rostriformis bugensis* (*D.r.b.*) at sites on the east (M-25, M-45) and west (H-8, B-7) of southern Michigan in 2004 and 2008. The AFDW/SL relationship is presented as $\log_e \text{AFDW} = b + a \log_e \text{SL}$. Also given is the AFDW of a standard 15-mm individual as derived from the determined relationship. Site depths: M-25 = 25 m, M-45 = 45 m, H-8 = 20 m, B-7 = 45 m.

Year/site	<i>b</i>		<i>a</i>		<i>R</i> ²		<i>n</i>		15-mm	
	<i>D.p.</i>	<i>D.r.b.</i>	<i>D.p.</i>	<i>D.r.b.</i>	<i>D.p.</i>	<i>D.r.b.</i>	<i>D.p.</i>	<i>D.r.b.</i>	<i>D.p.</i>	<i>D.r.b.</i>
2004										
M-25	−5.054	−6.545	2.658	3.195	0.752	0.883	148	150	8.53	8.20
M-45	−4.720	−6.674	2.394	3.174	0.828	0.936	149	148	5.23	6.83
H-8	−4.645	−5.695	2.343	2.726	0.850	0.934	94	94	5.47	5.40
B-7	−5.755	−6.995	2.933	3.426	0.892	0.947	93	99	8.91	9.80
Overall	−5.226	−6.535	2.651	3.143	0.776	0.874	484	491	7.05	7.21
2008										
M-25		−6.299		3.193		0.917		199		10.46
M-45		−5.469		2.659		0.928		193		5.65

were <5 mm. Mean density at the other eight sites in this interval was only 5857/m² (SE = 4055/m²). Except for a few individuals at Station H-13 (19 m depth), all individuals of *D. rostriformis bugensis* collected over the entire sampling period regardless of interval were the profunda phenotype.

In general, temporal trends in biomass paralleled those of density for both species. Mean biomass (AFDW) of *D. polymorpha* peaked in 2002 and then declined (Fig. 3). Maximum mean biomass was 6.3 g/m² at 31–50 m in 2002. For *D. rostriformis bugensis*, mean biomass increased beginning in 2001 and continued to increase at the three deepest depth intervals through 2008. Maximum mean biomass was 43.9 g/m² at 31–50 m in 2008. While trends in density and biomass were generally similar, there were some noted exceptions. The increase in mean density of *D. rostriformis bugensis* at 16–30 m between 2007 and 2008 was not accompanied by a corresponding increase in biomass. Also, while mean densities at 31–50 m were similar in 2007 and 2008, mean biomass was 31.8% higher in 2008 compared to 2007.

Year-to-year variation in size frequencies of both *D. polymorpha* and *D. rostriformis bugensis* at the different intervals is given in Table 1. Of interest are trends in the proportion of newly recruited individuals (<5 mm), since changes in the relative number of these small individuals would indicate whether recruitment in *D. polymorpha* and *D. rostriformis bugensis* varied with density shifts. For *D. polymorpha*, the mean percentage of individuals <5 mm in the total population was highly variable between years, ranging from 27.9% to 87.8% at 16–30 m, and from 2.9% to 69.8% at 31–50 m. In contrast, the mean proportion of individuals <5 mm in the *D. rostriformis bugensis* population was greater and less variable from year-to-year compared to *D. polymorpha*. At the two shallowest intervals, the mean proportion for *D. rostriformis bugensis* was 51.4% (coefficient of variation = 33.0%; *n* = 12), whereas the mean proportion for *D. polymorpha* was 34.4% (coefficient of variation = 71.5%; *n* = 15). The proportion of <5 mm individuals in the *D. rostriformis bugensis* population at depths deeper than 50 m was consistently greater than 65.0% and likely reflected the

recent expansion (since 2005) of the population into the offshore region. In 2008, mean size was 3.1 mm, 9.7 mm, 5.1 mm, and 3.9 mm at 16–30 m, 31–50 m, 51–90 m, and > 90 m, respectively.

Comparisons of nutritional state and physical characteristics: *D. polymorpha* and *D. rostriformis bugensis*

The nutritional state of both *D. polymorpha* and *D. rostriformis bugensis* (all profunda) were measured in 2004 when both species were present and relatively abundant. *D. rostriformis bugensis* had a higher condition index (CI) than *D. polymorpha* at each of the four sites sampled (H-8, B-7, M-25, and M-45), and this difference was significant ($F_{[1,1064]} = 229.56$, $P < 0.001$; Table 2). Overall, mean CI was 73.3 and 53.6, respectively, for the two species. Thus, on average, *D. rostriformis bugensis* had 36.5% more soft tissue relative to shell cavity volume than *D. polymorpha*. While the index was higher for *D. rostriformis bugensis* at each of the four sites, the relative difference was greater at 45 m than at 20–25 m. The difference between the shallow and deep depth was not significant ($F_{[1,1064]} = 0.312$, $P = 0.576$), but there was a significant depth × transect interaction ($F_{[1,1064]} = 397.296$, $P < 0.001$). At the Muskegon transect, both species had a higher CI at 25 m compared to 45 m, but at the Waukegan transect, both species had a higher value at 45 m compared to 20 m.

Differences between the two species were not as pronounced when AFDW–SL relationships were compared (Table 3). While regression slopes for the two species were significantly different (ANCOVA, $P < 0.01$), the difference between species was most evident at 45 m. Over all dates and sites, the weight of a standard 15-mm individual of *D. rostriformis bugensis* and *D. polymorpha* were comparable (7.21 mg vs. 7.05 mg).

Other physical characteristics of the shell and soft tissue of the two species are given in Table 4. Shell weight per unit shell length (SW/SL) was significantly lower in *D. rostriformis bugensis* ($F_{[1,1065]} = 207.5$, $P < 0.01$). Mean SW/SL for *D. rostriformis bugensis* was about one-half

Table 4

Mean (±SE) shell weight per shell length (SW/SL; mg per mm), tissue ash-free dry weight of tissue dry weight (%), and tissue dry weight of tissue wet weight (%) of *Dreissena polymorpha* (*D.p.*) and *Dreissena rostriformis bugensis* (*D.r.b.*) at sites on the east (M-25, M-45) and west (H-8, B-7) of southern Lake Michigan in 2004 and 2008. Site depths: M-25 = 25 m, M-45 = 45 m, H-8 = 20 m, B-7 = 45 m.

Year/site	SW/SL		AFDW of DW (%)		DW of WW (%)	
	<i>D.p.</i>	<i>D.r.b.</i>	<i>D.p.</i>	<i>D.r.b.</i>	<i>D.p.</i>	<i>D.r.b.</i>
2004						
M-25	14.2 ± 0.7	8.4 ± 0.4	65.7 ± 0.5	66.2 ± 0.6	7.1 ± 0.2	8.2 ± 0.2
M-45	16.5 ± 0.8	6.7 ± 0.3	67.4 ± 0.5	69.9 ± 0.6	3.9 ± 0.1	6.1 ± 0.1
H-8	10.0 ± 0.6	7.6 ± 0.5	65.8 ± 0.8	62.8 ± 1.0	4.2 ± 0.1	6.1 ± 0.2
B-7	20.7 ± 1.3	9.5 ± 0.7	64.8 ± 0.5	66.2 ± 0.6	6.3 ± 0.2	9.0 ± 0.2
2008						
M-25	–	9.0 ± 0.4	–	88.4 ± 0.6	–	7.5 ± 0.2
M-45	–	7.8 ± 0.3	–	87.8 ± 1.7	–	4.1 ± 0.1

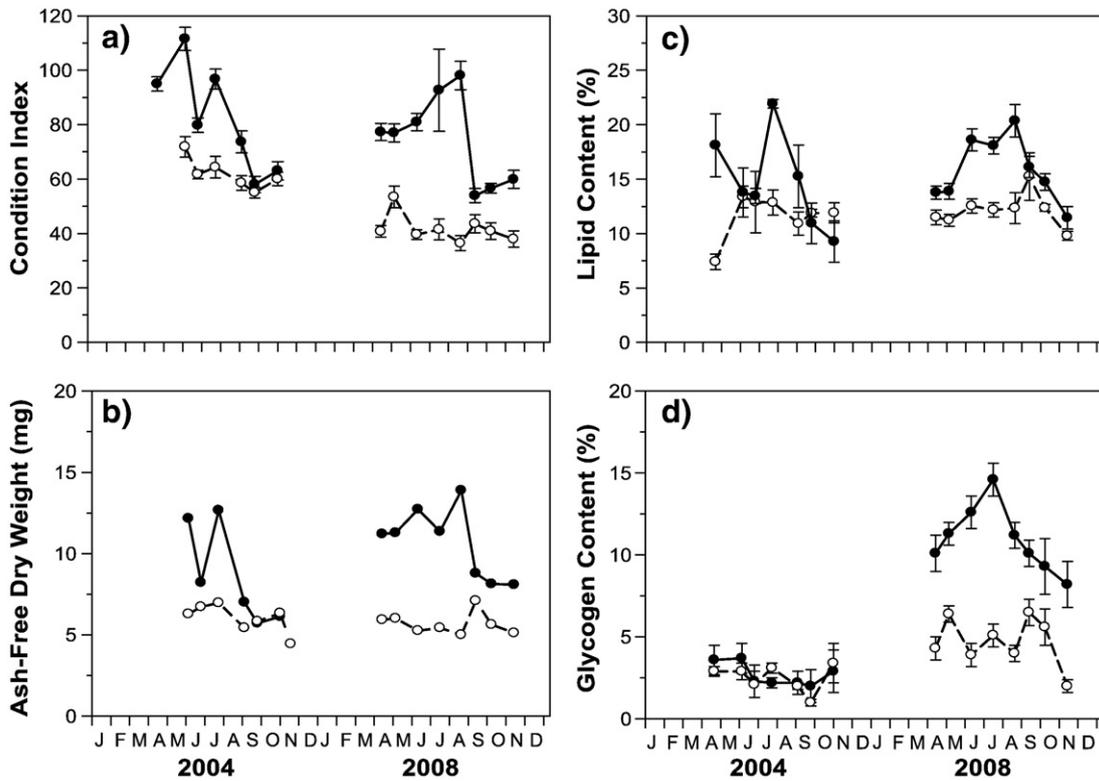


Fig. 4. Mean (\pm SE) condition index (a), ash-free dry weight (b), lipid content (c), and glycogen content (d) of *D. rostriformis bugensis* at M-25 (25 m) and M-45 (45 m) on each sampling date in 2004 and 2008. Values determined from various-sized individuals. Ash-free dry weight is given as the weight of a standard 15-mm individual determined from a AFDW/SL regression determined on each sampling date. M-25 = solid circle/solid line; M-45 = open circle/dashed line.

that of *D. polymorpha* (7.9 mg/mm vs. 15.3 mg/mm). The percentage of dry weight relative to wet weight (DW/WW; tissue only) was significantly higher in *D. rostriformis bugensis* ($F_{[1,1065]} = 171.2$, $P < 0.01$), but the percentage of ash-free dry weight relative to dry weight (AFDW/DW; tissue only) was not significantly different between the two species ($F_{[1,1065]} = 2.1$, $P \leq 0.15$).

D. rostriformis bugensis had a significantly higher lipid content than *D. polymorpha* ($F_{[1,155]} = 6.40$, $P = 0.012$) regardless of depth or transect (interactions were not significant, $P > 0.05$). Overall, mean lipid content of *D. rostriformis bugensis* was 12.4% compared to 10.7% for *D. polymorpha* (Table 2). Lipid content was significantly higher at 45 m compared to 20–25 m ($F_{[1,155]} = 25.664$, $P < 0.001$), but the depth \times transect interaction was also significant ($F_{[1,155]} = 59.174$, $P < 0.001$). As found for CI, overall lipid content of the two species was higher at the shallow site compared to the deeper site at the Muskegon transect but higher at the deeper site compared to the shallow site at the Waukegan transect. Mean glycogen content was 2.4% and 2.3% in *D. rostriformis bugensis* and *D. polymorpha*, respectively, and this difference was not significant ($F_{[1,164]} = 1.42$, $P = 0.25$; Table 2).

Yearly and seasonal trends in nutritional state of *D. rostriformis bugensis*

Given the rapid and continued expansion of the *D. rostriformis bugensis* population between 2004 and 2008 in the southern basin (Fig. 2), we were interested in relative changes in its nutritional state over this 4-year period. Densities at 25 m (M-25) and 45 m (M-45) over this period increased from 963/m² to 4212/m² and from 8748/m² to 13,023/m², respectively (Table 2). For both sampling depths, CI was significantly lower in 2008 than in 2004 ($F_{[1,735]} = 243.04$, $P < 0.001$), but this difference was depth-dependent (year \times depth interaction, $F_{[1,735]} = 58.68$, $P < 0.001$). The decline in CI between years was greater at 45 m than at 25 m (Table 2 and Fig. 4a). Consistent with a greater decline in CI at 45 m, the weight of a standard 15-mm individual at 45 m declined by 17% between the 2 years, but an increase was

evident at 25 m (Table 3). Slopes of the AFDW/SL regressions were significantly different between 2004 and 2008 at 45 m (ANCOVA, $P < 0.001$) but not at 25 m (ANCOVA, $P > 0.05$).

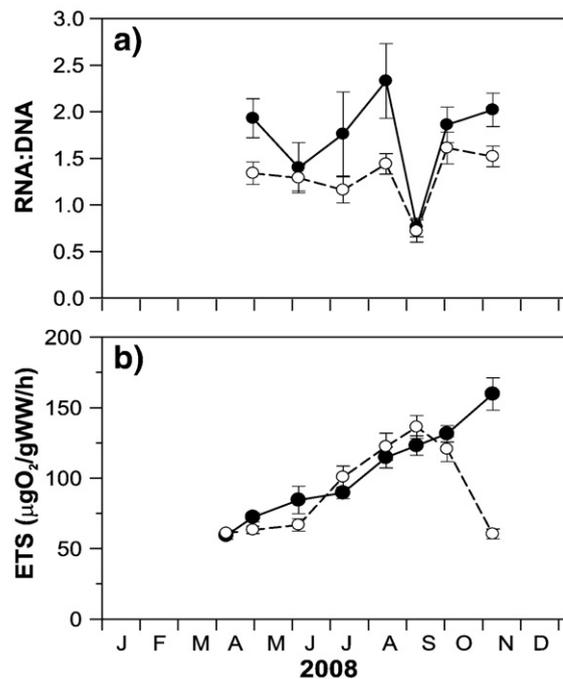


Fig. 5. Mean (\pm SE) RNA/DNA ratio (a) and ETS (b) of *D. rostriformis bugensis* at M-25 (25 m) and M-45 (45 m) on each sampling date in 2008. Values determined on various sized individuals on each sampling date. M-25 = solid circle/solid line, M-45 = open circle/dashed line.

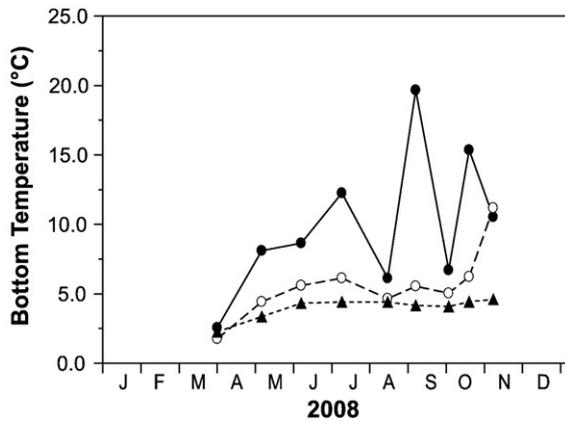


Fig. 6. Near-bottom water temperatures (°C) at M-25 (25 m), M-45 (45 m), and X-2 (93 m) on each sampling date in 2008. M-25 = solid circle/solid line; M-45 = open circle/dashed line; X-2 = solid triangle/dashed line.

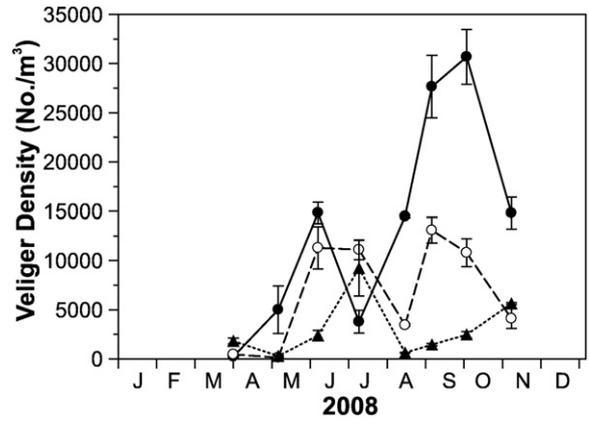


Fig. 7. Mean (\pm SE) density (no./m³) of *Dreissena veligers* at M-25 (25 m), M-45 (45 m), and X-2 (93 m) on each sampling date in 2008. M-25 = solid circle/solid line; M-45 = open circle/dashed line; X-2 = solid triangle/dashed line.

While relative tissue mass based on CI and AFDW/SL tended to be lower in 2008 compared to 2004, lipid content was not significantly different between the 2 years ($F_{[1,86]} = 1.84, P = 0.18$), and glycogen content was significantly higher in 2008 compared to 2004 ($F_{[1,86]} = 62.31, P < 0.01$; Table 2). When depths were compared across years, both lipid and glycogen content were significantly higher at 25 m than at 45 m (lipid: $F_{[1,86]} = 20.06, P < 0.01$; glycogen: $F_{[1,86]} = 8.28, P < 0.01$). For glycogen, however, there was a significant year \times depth interaction ($F_{[1,86]} = 10.56, P < 0.01$). Clearly, glycogen content was greater at 25 m than at 45 m in 2008 but not in 2004 (Table 2).

Seasonal trends in tissue mass and biochemical content varied between the two depths. At 25 m, CI and AFDW/SL remained generally high until late summer and then rapidly declined in both 2004 and 2008 (Figs. 4a and b). Seasonal trends in lipid content were similar; values remained relatively high until late summer and then declined in both years (Fig. 4c). However, a consistent seasonal pattern between the 2 years was not apparent for glycogen (Fig. 4d). Whereas a defined seasonal pattern was not apparent in 2004, trends in 2008 were similar to lipid content. Seasonal patterns of all indicators were different at 45 m compared to 25 m. CI was at a seasonal maximum in spring and then declined by late spring/early summer both years; AFDW/SL and lipid content followed this general seasonal pattern in 2004, but in 2008, a late summer increase was also evident (Fig. 4). As at 25 m, there were no consistent seasonal trends in glycogen at 45 m between the 2 years (Fig. 4d).

Since we measured RNA/DNA and ETS in *D. rostriformis bugensis* only in 2008, we could only compare the two depths over a seasonal period. RNA/DNA ratios were significantly lower at 45 m compared to 25 m (paired *t*-test, $P = 0.01; n = 7$). Ratios were lower at 45 m on each sampling date; overall mean ratios were 1.73 and 1.30 at 25 m and 45 m, respectively. Distinct seasonal patterns were not apparent except for the greatly lower ratios at both sites in early September

(Fig. 5a). In contrast, differences in ETS between the two depths were not significantly different (paired *t*-test, $P = 0.51; n = 8$). Values were generally similar at the two sites on each sampling date except in early November (Fig. 5b). On this date, mussels at 45 m had a mean ETS value that was 2.6 times lower than the mean value at 25 m. Otherwise, seasonal trends were similar at the two sites; values gradually increased from spring to fall. Mean ETS was 105.9 $\mu\text{g O}_2/\text{mg WW/h}$ at 25 m and 96.2 $\mu\text{g O}_2/\text{mg WW/h}$ at 45 m.

Seasonal patterns in water temperature, reproduction, and recruitment of D. rostriformis bugensis

Mean bottom temperature at 25 m (M-25) and 45 m (M-45) over all sampling dates in 2008 was 10.0 °C and 5.6 °C, respectively. Bottom temperatures at 25 m gradually increased in spring, but wide month-to-month fluctuations occurred between July and Sept after thermal stratification (Fig. 6). A maximum temperature of 19.7 °C occurred in early September. Bottom temperatures were more consistent at 45 m, ranging between 4.4 °C and 6.2 °C from early May to October. Maximum temperature at this site was 11.2 °C in early November. This November sampling date was the only time that bottom temperature at 45 m exceeded the bottom temperature at 25 m. Bottom temperatures at 93 m never exceeded 5.0 °C (Fig. 6).

The analysis of female reproductive state indicated distinctly different seasonal patterns in reproductive development and spawning of *D. rostriformis bugensis* at 25 m and 45 m in 2008 (Table 5). At 25 m, all females had mature oocytes on the first sampling date in early April, but these oocytes remained in the gonads through early August. Spent females were first noted in early September, and all females were spent by early November. All females at 45 m had mature oocytes by late April, but, unlike at 25 m, spent females were soon observed in early June, and all females were spent by early

Table 5

The percentage of female *D. rostriformis bugensis* at various stages of gametogenesis on each sampling date in 2008. See text for explanation of each stage. Site depths: M-25 = 25 m, M-45 = 45 m.

Date/stage	M-25					M-45				
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Apr 08	0.0	0.0	0.0	100.0	0.0	0.0	16.7	41.7	41.7	0.0
Apr 29	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0
Jun 04	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	40.0	60.0
Jul 09	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	40.0	60.0
Aug 12	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0
Sep 04	0.0	0.0	0.0	80.0	20.0	0.0	0.0	0.0	0.0	100.0
Sep 30	0.0	0.0	0.0	60.0	40.0	0.0	0.0	0.0	0.0	100.0
Nov 04	50.0	0.0	0.0	0.0	50.0	70.0	0.0	0.0	0.0	30.0

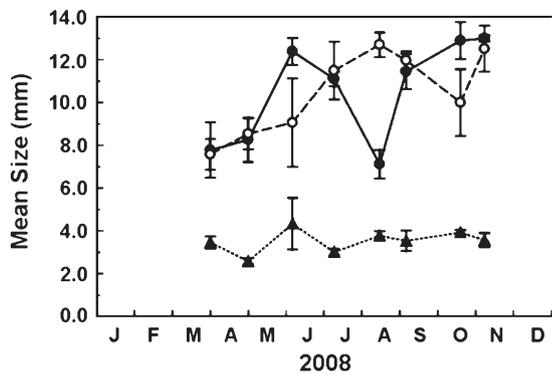


Fig. 8. Mean size (\pm SE) of *D. rostriformis bugensis* at M-25 (25 m), M-45 (45 m), and X-2 (93 m) on each sampling date in 2008. M-25 = solid circle/solid line; M-45 = open circle/dashed line; X-2 = solid triangle/dashed line.

August. Thus, spawning at 25 m occurred after early August and was complete by November, whereas spawning at 45 m occurred before early June and was complete by early August. As indicated by the percentage of females with early stage oocytes, a new reproductive cycle was initiated at both depths after late September.

Coincident with seasonal spawning patterns at the two depths, veliger densities showed two distinct seasonal peaks, one in spring (early June) and the other in fall (September/October) (Fig. 7). At 25 m, the fall peak was 2 times greater than the spring peak ($35,000/\text{m}^3$ vs. $15,000/\text{m}^3$), but at 45 m, the fall and spring peaks were similar ($12,000/\text{m}^3$ vs. $13,000/\text{m}^3$). Given one spawning event at each site and, since spawning at 25 m occurred in late summer and spawning at 45 m occurred in spring, the source of the veliger peak at 25 m in spring was likely from spawned individuals at deeper depths, and the source of the veliger peak at 45 m in fall was likely from spawned individuals at shallower depths. Veliger densities were also determined at 93 m (site X-2). Densities at this depth peaked in early July (Fig. 7). Since we did not determine the reproductive state of females at this site, the July peak could have resulted from spawned individuals at the site itself, or from veligers that had been transported offshore from spawned females in shallower regions (i.e., 45 m).

Seasonal changes in the relative number of newly settled recruits, as defined by the proportion of individuals <5 mm, did not closely coincide with spawning and veliger peaks. A relatively high percentage of these small individuals occurred in early spring (April) at both 25 m (up to 33%) and 45 m (50%), which was before spawning at both depths. Thereafter, the percentage was generally less than 14% of the population through the rest of the seasonal period. The exception was the high percentage (43%) at 25 m in early August. This high percentage was not likely related to reproductive activity since it occurred before spawning at this depth. There was no significant difference in mean size of individuals at 25 and 45 m (paired *t*-test, $P=0.99$; $n=7$). In general, mean size increased from April to November at both depths, with the noted exception of a low mean size at 25 m in August (Fig. 8). Mean size was lowest in early April (7.7 mm) and highest in early November (12.8 mm). Mean size at 93 m was less than 4 mm regardless of season; 77–92% of the population consisted of individuals <5 mm over all sampling dates.

Discussion

D. rostriformis bugensis began to colonize the southern basin of Lake Michigan in 2001 and has progressively expanded from relatively shallow (<50 m) to deeper (>50 m) depths ever since. Based on 2008 results, continued population expansion within the former depth region, as indicated by increased density and biomass, was not well defined. At 16–30 m, mean densities continued to increase through 2008, but biomass has not increased since 2004. The

population in this depth interval is consistently dominated by small individuals (<5 mm) that are highly patchy in distribution, perhaps indicating that individuals are settling but not able to grow in this unstable, nearshore habitat. At 31–50 m, mean densities were relatively unchanged between 2006 and 2008, but biomass continued to increase; it was 32% higher in 2008 compared to 2007. This can be attributed to a maturing population at this depth interval, as mean size was greater in 2008 than in any previous year. Indeed, there was a greater percentage of individuals larger than 15 mm in 2008 compared to 2007 (24.8% vs. 16.0%). Although this percentage increase was relatively minor, these larger individuals accounted for 92% of the biomass increase at this depth interval between the 2 years. At the very least, these examples emphasize the importance of obtaining biomass in addition to densities when assessing trends over time.

Based on trends in both density and biomass, the population at depths >50 m will likely continue to expand. Mean density at 51–90 m in 2008 was already similar to that found at 31–50 m, although the population only began to expand at this interval in 2005. Biomass at this interval was less than at 31–50 m, but the average size of an individual was much smaller (5.1 mm vs. 9.7 mm), indicating that biomass will likely increase at this interval as the population matures. With the average size of an individual even smaller in the >90 -m interval, increases in biomass at this interval may also be expected.

Although dreissenid densities in the Great Lakes have been widely reported, few studies have determined biomass, thus limiting our comparisons. Mean biomass (AFDW) of *D. polymorpha* on hard substrates in inner Saginaw Bay peaked at $61.9 \text{ g}/\text{m}^2$ in 1992 before the population declined (Nalepa et al., 2003). In Lake Erie, mean biomass (tissue DW) of dreissenids was 5.8, 14.2, and $104.7 \text{ g}/\text{m}^2$ in the west, central, and eastern basins in 2002 (Patterson et al., 2005). For a more realistic comparison between our Lake Michigan biomass estimates and those in Lake Erie, we restricted the Lake Erie estimates to those found in the deeper region of the eastern basin where the dreissenid population consisted entirely of *D. rostriformis bugensis*, and where environmental conditions (i.e., soft sediments, hypolimnetic temperatures) were most similar to offshore, southern Lake Michigan. Mean biomass (DW) at sites >24 m in the eastern basin (maximum depth 64 m) was $38.6 \text{ g}/\text{m}^2$ (Patterson et al., 2005). At the generally comparable depth of 31–50 m in Lake Michigan, mean biomass in 2008 was $49.9 \text{ g}/\text{m}^2$ (assuming AFDW is 88% of dry weight as found in this study), which compares well with the eastern basin value.

A recent study in Lake Michigan reported trends in wet weight biomass of dreissenids (shell and soft tissue) at different depth intervals between 1999 and 2007 (Bunnell et al., 2009). In 2007, mean total wet biomass at 9–30 m, 31–60 m, 61–90 m, and 91–110 m was 0.1, 4.4, 10.3, and $11.2 \text{ g}/\text{m}^2$, respectively. If we convert mean AFDW biomass found in 2007 in our study to whole wet weight, we obtain estimates of 62.7, 378.9, 92.8, and $1.0 \text{ g}/\text{m}^2$ at our four depth intervals (16–30 m, 31–50 m, 51–90 m, >90 m). Thus, although depth categories were generally similar, our estimates were vastly different from those of Bunnell et al. (2009). These authors collected dreissenids with a fish trawl and recognized that their biomass estimates were biased low. Interestingly, while our wet biomass estimates were 9–300 times higher at the three shallowest intervals, they were 10-fold lower at the deepest interval. We suspect this lower estimate may simply be a result of delayed colonization in the deep region of the southern basin compared to the estimate of Bunnell et al. (2009), which was for the entire lake. In 2008, our total wet biomass at >90 m increased to $10.7 \text{ g}/\text{m}^2$, a value more comparable. Regardless, it seems apparent that while trawl collections may be useful in determining relative abundances of the various dreissenid species, or shifts in spatial distributions (Mills et al., 1999; French et al., 2007; Bunnell et al., 2009), they should not be relied upon to estimate population density or biomass.

The gradual expansion of *D. rostriformis bugensis* into offshore regions as found in southern Lake Michigan is not unique to the Great Lakes; similar expansions have been documented in Lake Ontario (Watkins et al., 2007) and in Lake Huron (French et al., 2009). However, the *D. rostriformis bugensis* population in southern Lake Michigan seems to be increasing at a comparatively rapid rate. For example, *D. rostriformis bugensis* was first found in Lake Ontario in the early 1990s and has been expanding ever since (Mills et al., 1993, 1999; Watkins et al., 2007). In the most recent survey conducted in 2003, which was about 10 years after the population became established, mean density was 8106/m² at 31–90 m (Watkins et al., 2007). Mean density in southern Lake Michigan at 31–90 m in 2008 was 13,800/m² after just 7 years. Further, *D. rostriformis bugensis* became established in Lake Huron at the same time as in Lake Michigan (Nalepa et al., 2001), but mean density at 31–90 m in 2007 was only 1173/m² (Nalepa, unpublished data).

The decline and virtual disappearance of *D. polymorpha* as *D. rostriformis bugensis* expanded in the southern basin follows a pattern reported previously throughout Lake Michigan (Nalepa et al., 2009) and in other Great Lakes (Mills et al., 1999; Patterson et al., 2005; Wilson et al., 2006). Laboratory experiments have shown that, compared to *D. polymorpha*, *D. rostriformis bugensis* has a lower respiration rate at various seasonal temperatures (Stoeckmann, 2003) and a higher assimilation efficiency, especially at low food concentrations (Baldwin et al., 2002). These attributes allow *D. rostriformis bugensis* to allocate less energy to metabolic maintenance and more to growth and reproduction. The two most consistent differences between the two species in our study was the amount of tissue mass within the shell cavity (CI), and the shell weight per unit shell length (SW/SL). Overall, mean CI for *D. rostriformis bugensis* was 27% higher, and mean SW/SL was 48% lower relative to *D. polymorpha*. These differences suggest that, under exactly the same environmental conditions, *D. r. bugensis* allocates greater amounts of energy to tissue mass relative to shell mass. With tissue loss a common occurrence in dreissenids subjected to nutritional stress (Sprung and Borcharding, 1991), *D. rostriformis bugensis* would be better suited to survive under low food conditions.

The nutritional state of *D. polymorpha* in 2004 was generally similar to values found in other regions of the Great Lakes where populations were in the state of decline. The most commonly used indicator of dreissenid condition in the Great Lakes has been the tissue weight–shell length relationship (Nalepa et al., 1993, 1995; Chase and Bailey, 1999a; Vanderploeg et al., 2009). Based on the four sites sampled in 2004, tissue weight of a standard 15-mm *D. polymorpha* in fall was 6.2 mg (range 5.1–7.2 mg). Fall is considered the best time to assess this indicator since spawning has occurred, and values more realistically reflect baseline tissue weight. When populations peaked and were declining in Saginaw Bay, a 15-mm individual weighed 3.1 mg (Nalepa et al., 1995), and in Lake St. Clair, the weight of a 15-mm individual within a declining population was 5.8–9.1 mg (Nalepa et al., 1993). For perspective, tissue weight of a 15-mm *D. polymorpha* is generally over 8 mg when food is not limiting (Nalepa et al., 1995).

Although numerous factors can influence size structure of dreissenid populations including growth, reproduction, and size-specific predation, trends in size structure relative to density can provide insights into the mechanisms for population shifts. In general, during the early stages of dreissenid colonization, the population is dominated by small individuals, but over time, mean size increases as the population matures and several year-classes become present (Nalepa et al., 1995). Food requirements per unit of tissue mass increases with size of the individual (Walz 1978a,b; Wacker and von Elert, 2008), so an increasing mean size after initial colonization suggests adequate food resources. Conversely, a decreasing mean size suggests limited food availability. Mean size of *D. polymorpha* declined along with density as chlorophyll levels declined in Lake St. Clair (Nalepa et al., 1996). Also, mean size of *D. rostriformis bugensis*

increased, and that of *D. polymorpha* declined, as the former species replaced the latter in Lake Erie (Mills et al., 1999). Since size structure at 16–30 m was highly variable from year-to-year and likely influenced by physical disturbances (wave action, unstable substrates), the 31- to 50-m interval was better suited to examine trends in size structure relative to density. For *D. polymorpha*, there was a good correlation between mean size and density (Pearson correlation coefficient = 0.71) over the period 1998–2005 when this species was still present. Along with density, mean shell length increased to a peak in 2001–2002 and then declined. Increasing mean size would indicate that the population was still growing at a good rate at the time *D. rostriformis bugensis* began to colonize this depth interval in 2001–2002. Filtering capacity of the dreissenid population at this depth interval in 2001–2002 was still below algal growth rates suggesting adequate food resources (Vanderploeg et al., 2010). A decline in mean size along with decreased densities after 2001–2002 suggests that larger individuals were disappearing from the population, consistent with decreased food availability. For *D. rostriformis bugensis*, mean size and density were not as well correlated (Pearson correlation coefficient = 0.51) over the period 2002–2008, suggesting slow growth rates relative to increased density. During this 6-year period, the increase in mean size of the *D. rostriformis bugensis* population at 31–50 m was only 0.6 mm/year. In comparison, mean size of the *D. polymorpha* population between 1998 and 2001 at the same depth interval was 1.9 mm/year. During the early colonization period between 1992 and 1995 in a shallow region of eastern Lake Erie (<30 m), mean size of *D. rostriformis bugensis* increased 1.3 mm/year (Mills et al., 1999). Although growth rates at 31–50 m were relatively low, mean size of the population was still increasing in 2008, seemingly indicating that the population was not yet food limited.

Depths of the two sites where dreissenids were collected for documenting seasonal reproductive activity and nutritional state (25 m and 45 m) were specifically located above and below the thermocline to examine the influence of different temperature regimes. Temperature has a strong, direct influence on seasonal reproductive activity in *D. polymorpha* (Sprung, 1987; Nichols, 1996), but little is known of temperature impacts on reproductive activity in *D. rostriformis bugensis*. The seasonal timing of gametogenesis and spawning in *D. rostriformis bugensis* at the two depths was indeed different, but trends relative to temperature varied from what we expected. At 45 m, all females had mature oocytes in late April and spawning began soon after when bottom temperatures were at 4.5–6.0 °C. These findings agree with others that show spawning of *D. rostriformis bugensis* occurs in spring in deeper regions. For instance, Claxton and Mackie (1998) found that *D. rostriformis bugensis* profunda spawned in late June at 23 m when temperatures reached 9 °C in eastern Lake Erie, and Roe and MacIsaac (1997) collected spawned and gravid (with mature oocytes) females in mid-July (their only sampling date) at 37 m and 55 m when water temperatures were 4.8–6.0 °C. Although the change was minor, potentially the temperature increase at our 45-m site from a winter low of 2 °C to 4.5–6.0 °C could have triggered spawning. In contrast, spawning at 25 m did not occur until late summer despite females having mature oocytes since early April. Temperature warmed first at 25 m, thus, if an increase in temperature initiated spawning, we should have observed spawning first at this depth. Either absolute increases in temperature had little impact as a spawning trigger, or other environmental cues were more important. Ram and Nichols (1993) argued that an increase in temperature may provide favorable conditions to spawn, but does not trigger it. Rather, chemicals associated with phytoplankton are the key triggering mechanism. As argued, temperatures can vary erratically, while phytoplankton increases are generally gradual and integrate environmental conditions (including temperature) that favor successful mussel recruitment. It is difficult to offer a scenario where phytoplankton cues were present earlier at 45 m compared to 25 m, but bottom temperatures at 25 m fluctuated broadly and

conceivably may have delayed spawning relative to 45 m. Temperature stability can have a strong impact on spawning (Ram and Nichols, 1993). Regardless, it is apparent that environmental cues that trigger spawning at these depths in Lake Michigan need further investigation.

Based on the evidence, reproductive effort was greater at 25 m than at 45 m. The amount of reproductive material can be estimated from the change in tissue mass before and after spawning (Nalepa et al., 1993; Chase and Bailey, 1999b). Since Claxton and Mackie (1998) found that individuals of *D. rostriformis bugensis* greater than 8 mm were mature and capable of spawning, we assume that all individuals in our study for which we measured nutritional state and biochemical content (smallest size = 9 mm) were also capable of spawning. In 2008, tissue dry mass as measured by CI declined by 41% after spawning at 25 m, compared to a decline of 26% at 45 m. Post-spawning declines in AFDW/SL and lipid content were also greater at 25 m. Overall, percentage declines in tissue mass are within the range of 20–45% for *D. polymorpha* in shallower waters (Sprung and Borcharding, 1991; Sprung, 1995), and also the 20–40% decline observed in a number of marine mussel species that have a pelagic life stage (summarized in Sprung, 1995). The greater reproductive effort at 25 m was evident when the peak in the number of postspawn veligers was determined on an areal basis and examined relative to benthic adult biomass. Veliger numbers peaked at 336,500/g AFDW at 25 m compared to 13,300/g AFDW at 45 m.

The seasonal timing of spawning at the two depths varied (i.e., late summer at 25 m, spring at 45 m), and although peaks in veliger densities coincided with spawning at both depths, spawning at one depth also led to increased veliger densities at the other. This suggests considerable horizontal mixing of veligers across depth regions. In large lakes, hydrodynamic forces such as longshore currents and upwelling/downwelling are an effective mechanism by which veligers get transported from nearshore to offshore and also from offshore to nearshore (Wacker and von Elert, 2003). There has been some uncertainty as to whether *D. rostriformis bugensis* recruits in deeper regions originated from local adults, or originated from adults in shallower regions and were transported offshore (Roe and MacIsaac, 1997; Martel et al., 2001). The spring spawning at 45 m and the subsequent increase in veligers at both 45 m and 25 m suggests that adults found in deeper waters contribute not only to local recruitment but also to recruitment in shallower water.

A clear increase in new recruits relative to seasonal reproductive events was not apparent. The greatest proportion of individuals < 5 mm occurred in early spring at both sites in 2008, suggesting that these spring recruits were the previous year's cohort that had settled over winter. Recruitment can be highly variable in dreissenids and may not necessarily be synchronous with spawning activity or related to veliger abundances (Sprung, 1989; Chase and Bailey, 1999a; Wacker and von Elert, 2003).

Based on differences in the various indices of condition, the nutritional state of *D. rostriformis bugensis* was lower at 45 m than at 25 m for the Muskegon sites. That is, CI, AFDW/SL, and lipid content were lower at 45 m in both 2004 and 2008, and growth, as measured by RNA/DNA in 2008, was also lower. Seemingly inconsistent with these results, however, were the higher values of some indices at 45 m compared to 20 m off Waukegan in 2004. We suspect this different spatial pattern was a function of relatively low dreissenid densities and hence higher food availability at the Waukegan 45-m site. In 2004, total dreissenid density at this site was only 600/m² compared to 9700/m² at the 45-m site off Muskegon. In 2008, mean density at the former site increased to 9060/m²; unfortunately, nutritional state was not measured at the Waukegan sites in 2008.

Lower nutritional state of *D. rostriformis bugensis* at 45 m compared to 25 m was likely a function of lower food availability at the former depth. Mean chlorophyll in 2008 at 45 m was 1.3 µg/L compared to 2.5 µg/L at shallower depths (Vanderploeg et al.,

2010). Further, biomass was consistently greater at 31–50 m compared to 16–30 m (Fig. 3), leading to greater competition for food resources. Greater biomass at the former interval despite lower food availability can best be explained by physical features of the nearshore/offshore environment in this region of Lake Michigan. Before dreissenid colonization, biomass of the benthic community was greater at 31–50 m, and this spatial pattern was attributed to unstable bottom conditions at shallower depths (Mozley and Winnell, 1975; Nalepa, 1989). Above the thermocline, wave-induced sediment resuspension and temperature fluctuations create a harsh environment for benthic populations and limit numbers. We suspect these same physical features were limiting *D. rostriformis bugensis* populations at our 25-m site. These features would affect population size through diminished survival, but have minimal impact on nutritional state; that is, mussels that survive these harsh conditions would likely not be food limited.

Given high standing stocks at 31–50 m, does the nutritional state of *D. rostriformis bugensis* at 45 m indicate that the population is nearing self-limitation? The various indices provided a mixed interpretation. Both indices based on tissue mass, CI and AFDW/SL, were lower in 2008 compared to 2004, but lipid content was little changed between years, and glycogen was higher. The increase in 2008 for glycogen was a result of atypically low values found in 2004, which did not vary with season or depth as found in 2008. In laboratory experiments with *D. polymorpha*, glycogen levels did not decline over a period of starvation as did lipid content and tissue weight (Frischer et al., 2000), suggesting glycogen content may not be a good indicator of nutritional stress in *D. rostriformis bugensis*. Comparisons of studies assessing the relationship between food availability and nutritional response in *D. polymorpha* showed that both lipids and carbohydrates are more variable and inconsistent relative to tissue mass as indicators of nutritional stress (Nalepa et al., 1993). The tissue weight of a 15-mm *D. rostriformis bugensis* at 45 m in 2008 was 5.7 mg, a value below the 8.1 mg and 12.1 mg found for a 15-mm *D. rostriformis bugensis* at 37 m and 55 m in eastern Lake Erie (Roe and MacIsaac, 1997), and comparable to the 5.2 mg value found for a 15-mm *D. polymorpha* at this depth in 2004. Thus, based on changes in relative tissue mass, *D. rostriformis bugensis* may indeed be nutritionally stressed. Since *D. rostriformis bugensis* began to expand in this region of Lake Michigan, spring chlorophyll levels declined from over 4 µg/L to near 1 µg/L in offshore waters, and the spring diatom bloom is no longer evident (Fahnenstiel et al., 2010). Diatoms are a rich food source, and isothermal mixing in the spring gives bottom-dwelling dreissenids access to diatoms throughout the entire water column. Despite the relatively low nutritional state of the individual based on tissue mass, the population in 2008 was still in a state of expansion as indicated by the continued increase in population biomass and mean size. Seemingly, there is a different feedback mechanism between nutritional state of the individual and population growth for *D. rostriformis bugensis* as compared to *D. polymorpha*, which may be expected given its higher assimilation efficiency and lower respiration rate (Baldwin et al., 2002; Stoekmann, 2003).

Spatial and seasonal differences in the RNA/DNA ratio and ETS are difficult to interpret because these variables have not been well studied in dreissenids. While the amount of DNA is fixed within a cell, the amount of RNA is a function of somatic growth, and the ratio will vary depending on such factors as food availability, temperature, and life stage (Chicharo and Chicharo, 2008). The ratio was greater at 25 m compared to 45 m, indicating growth was greater at 25 m, which is consistent with other indices indicating nutritional state was better at the shallower depth. The ratio varied little over a seasonal period, except for the unexplained large decline at both sites in early September. Growth in dreissenids declines during the spawning period (Sprung, 1995). However, while the September decline occurred during the time of spawning at 25 m, it did not occur at

the time of spawning at 45 m. The otherwise seasonally stable ratio at both depths suggests that growth is consistent throughout the year and uninfluenced by seasonal changes in temperature or reproductive activity. While both temperature and food availability can influence dreissenid growth (Walz 1978a,b; Sprung, 1995; MacIsaac, 1994; Wacker and von Elert, 2008), the lack of seasonal changes in the ratio at both depths suggests that the lower ratio at 45 m was a result of lower food availability rather than lower temperatures compared to 25 m. Excluding the atypical values in early September, the mean RNA/DNA ratio for *D. rostriformis bugensis* was 1.9 and 1.4 at 25 and 45 m, respectively. Without controlled experiments to assess changes in ratios relative to food conditions, the relevance of these low values is not clear. In many organisms, values <1 suggest nutritional stress and diminished survival (Chicharo and Chicharo, 2008). In laboratory experiments, Schlechtriem et al. (2008) examined the RNA/DNA ratios in starving *Mysis* and found values <1.5–1.8 were indicative of metabolically stressed animals; ratios of *in situ* animals were generally greater than 2. If anything, relatively low ratios in *D. rostriformis bugensis* would suggest that growth was minimal, consistent with the slow growth rate of the population based on size structure as discussed previously.

The assay for ETS activity estimates the potential respiratory capacity of the organism by measuring the enzymatic activity of the rate-limiting step in oxygen utilization for adenosine triphosphate (ATP) production. For ETS, this step is the oxidation of the coenzyme UQ-cytochrome *b* complex (Broberg, 1985). The assay has been widely used to measure the oxygen consumption potential of seawater, sediments, phytoplankton, and zooplankton (Packard, 1971; Packard et al., 1971; Jones and Simon, 1979). Since an organism's ETS activity requires several days to weeks to adjust to changes in environmental conditions, it is less subject to experimental artifacts and short-term fluctuations when compared to direct measurements of respiration (Bämstedt, 1980; Cammen et al., 1990).

Similar to the RNA/DNA ratio, seasonal changes in ETS were not related to reproductive activity at either 25 m or 45 m. Rather, the gradual increase in ETS from spring to fall at both depths suggests that temperature was the driving variable for this seasonal pattern. In dreissenids, there is a strong relationship between ETS/respiration and water temperature (Fanslow et al., 2001; Stoeckmann, 2003). Yet ETS at 25 m and 45 m was similar, despite the generally higher temperatures at 25 m. Short-term, temperature fluctuations were far greater at 25 m compared to 45 m and, since ETS is a long-term metabolic response to environmental conditions, these short-term fluctuations may have had a negative influence on ETS. ETS was actually greater at 45 m during the stratified period (July–September) when temperature fluctuations at 25 m were most severe. Similarly, the dramatic, one-time decline in ETS at 45 m occurred in November when temperatures were twice that found on any other sampling date. Interestingly, the RNA/DNA ratio (i.e., growth) did not increase with seasonal temperature increases while ETS (i.e., respiration) did. Hence, it seems that *D. rostriformis bugensis* can regulate food intake or assimilation efficiency to maintain growth in lieu of higher metabolic costs. Assimilation efficiency in *D. rostriformis bugensis* can be highly variable (Baldwin et al., 2002).

ETS for *D. rostriformis bugensis* in southern Lake Michigan was 2–3 times less than for *D. polymorpha* in outer Saginaw Bay at similar temperatures. Over the entire seasonal period in Lake Michigan, mean ETS was 7.94 $\mu\text{g O}_2/\text{mg DW/h}$ ($\text{DW} = 7.1\% \text{ WW}$) and mean temperature was 10.0 °C at 25 m, while mean ETS was 3.94 $\mu\text{g O}_2/\text{mg DW/h}$ ($\text{DW} = 4.1\% \text{ WW}$) and mean temperature was 5.6 °C at 45 m. From an ETS/temperature regression derived from two seasonal periods in outer Saginaw Bay (Fanslow et al., 2001), comparable ETS values for *D. polymorpha* were 15.1 $\mu\text{g O}_2/\text{mg DW/h}$ at 10.0 °C and 9.6 $\mu\text{g O}_2/\text{mg DW/h}$ at 5.6 °C. Although we did not measure respiration (*R*) directly, it can be approximated from the relationship between ETS and *R*, which is generally consistent for a given

taxonomic group and a function of life mode, with more active organisms having higher ratios (Cammen et al., 1990). For *D. polymorpha* in Saginaw Bay, the ratio varied between 0.02 and 0.09 over a seasonal period (Fanslow et al., 2001), and in laboratory experiments, the ratio for *D. polymorpha* was 0.06–0.07 regardless of individual size (Madon et al., 1998). Since both *D. rostriformis bugensis* and *D. polymorpha* have a similar sessile life mode, we assume a comparable ETS/*R* ratio of 0.06 for *D. rostriformis bugensis*. From this ratio, mean respiration rates were estimated to be 0.48 $\mu\text{g O}_2/\text{mg DW/h}$ and 0.23 $\mu\text{g O}_2/\text{mg DW/h}$ at 25 m and 45 m, respectively. These values are far less than the 1.45 $\mu\text{g O}_2/\text{mg DW/h}$ and 0.95 $\mu\text{g O}_2/\text{mg DW/h}$ at comparable temperatures (10.0 °C and 5.0 °C) reported for *D. rostriformis bugensis* from Lake Erie (Stoekmann, 2003). The phenotype of *D. rostriformis bugensis* in the Lake Erie study was not given but was likely the epilimnetic phenotype since the mussels were collected from a depth of only 4–5 m in the shallow western basin. Our lower estimates of respiration rates for the profunda phenotype suggest that this phenotype has lower metabolic costs and is thus better suited to the low food availability found in offshore waters compared to the epilimnetic phenotype. Also, it suggests that the profunda phenotype will more effectively outcompete *D. polymorpha* and potentially explains its rapid displacement of *D. polymorpha* in southern Lake Michigan.

As discussed by Nalepa et al. (2009), the expansion of *D. rostriformis bugensis* in southern Lake Michigan represents a major increase in benthic biomass and a disruption in the flow of nutrients/energy through the food web. Mean biomass (AFDW) of the benthic community in the southern basin before dreissenid introduction (i.e., 1980 and 1981) was 4.9, 7.8, 4.2, and 1.9 g/m^2 at 16–30 m, 31–50 m, 51–90 m, and > 90 m (Nalepa, 1989). When weighted for depth over the entire basin, total mean benthic biomass in the early 1980s was 4.0 g/m^2 . In 2008, depth-weighted biomass of just *D. rostriformis bugensis* was 10.9 g/m^2 , over a 2.5-fold increase. With nutrient loads declining in Lake Michigan between the early 1980s and 2008 (Mida et al., 2010), carrying capacity for biological productivity in the lake has declined, and therefore, the increase in benthic biomass must have come at a cost to other components of the food web. For one, the benthic amphipod *Diporeia* has been steadily declining in Lake Michigan since the introduction of dreissenids (Nalepa et al., 2009). Depth-weighted biomass of *Diporeia* in 1980–1981 was 2.5 g/m^2 , so even the near complete loss of *Diporeia* from the southern basin can only account for a portion (36%) of the mass now represented in *D. rostriformis bugensis* standing stocks. Further, the increase in benthic biomass does not take into account dreissenid shell mass. Shells take energy to produce but have no energetic value to other trophic levels. Shell material accounted for 91% of dry mass of *D. rostriformis bugensis* in 2008 (all dates at both sites), so total depth-weighted mean standing stock of just shell material in the southern basin was 121 g/m^2 . This represents energy totally lost to the food web at any given point in time.

Some fish species feed on dreissenids and may derive some benefits (Pothoven and Nalepa 2006; Madenjian et al. 2010), but direct and indirect negative impacts of dreissenids on fish have been widely reported (Mohr and Nalepa 2005; Hondorp et al., 2005; Madenjian et al., 2006). Coincident with the expansion of *D. rostriformis bugensis*, prey fish biomass in Lake Michigan declined 71% between 2003 and 2007 (Bunnell et al., 2009) and may represent a majority of the remaining energetic trade-off for the increase in *D. rostriformis bugensis* standing stocks. While it was argued that the decrease in prey fish biomass was more coincidental than mechanistic relative to the expansion of *D. rostriformis bugensis* (Bunnell et al., 2009), continued examination of trends will provide further insights into direct and indirect relationships between *D. r. bugensis* and fish populations. As of 2008, the *D. rostriformis bugensis* population at depths <50 m in southern Lake Michigan was becoming more stable but not decreasing, while the population at >50 m was still expanding. The population at the latter depth interval was dominated by small

individuals. While conceivably these individuals may be somewhat food-limited because of recent decreases in pelagic productivity in the offshore (Fahnenstiel et al., 2010), we suspect that future increases in biomass will occur as the population matures, and continued impacts on other food web components can be expected.

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