Temporal Trends in Nutritional State and Reproduction of Quagga Mussels (*Dreissena rostriformis bugensis*) in Southern Lake Michigan

By

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ABSTRACT:

Currently little is known about the nutritional state and spawning patterns of quagga mussels (*Dreissena rostriformis bugensis*) in deep regions of the Great Lakes. This lack of information severely limits our ability to predict the future ecological impacts of *D. r. bugensis* throughout the Great Lakes and other large, deep lakes. To address these issues indices of nutritional state and spawning patterns were determined at monthly intervals (April-September) at three established sites along a depth transect (25, 45, and 93 m) in Southern Lake Michigan in 2013. These indices were compared to values at the 25-m and 45-m sites in 2004 and 2008. In addition, density and AFDW biomass were measured seasonally in March, July, and August at the same three sites.

Condition index (CI), a ratio of dry soft tissue weight to internal shell capacity, was used to assess nutritional state and a gametogenic index was used to monitor reproductive activity. In 2013 annual mean CI was 56.6, 32.4, and 37.9 at 25, 45, and 93 m, respectively. Densities at 25 m (8811/m^2) and 45 m (8796/m^2) were similar however biomass was higher at 45 m (53.65 g/m^2) than at 25 m (35.98 g/m^2). Density was highest at 93 m (12152/m^2) but biomass was lowest at this depth (8.22 g/m^2). In terms of spawning patterns, mussels at 25 m had not yet spawned by September. Reproductive patterns were more variable at 45 m than at 25 m; however, spawning began earlier at 45 m with half or more mussels being spent beginning in July; at 93 m spawning began in August.

Results indicate that competition for food was high, particularly at intermediate depths where biomass was greatest, and that this competition along with decreasing chlorophyll *a* concentrations in the southern basin of Lake Michigan likely caused decreases in nutritional state. At 45 m and 93 m CI decreased with increasing shell length; this also suggested that food availability may be limiting for larger individuals. A comparison of this study to earlier studies indicated that the population of *D. rostriformis*

bugensis continues to grow and expand into deeper offshore regions of Lake Michigan as based upon an increase in biomass and mean size, along with evidence of reproduction at 93 m. Although expansion continues into deeper regions, results indicate that growth is stabilizing at intermediate depths as densities and biomass appear to be stabilizing and CI is in decline. Densities and CI continue to decline at 25 m. If populations continue to decline in intermediate and shallow regions the negative effects that *D. r. bugensis* has had on the Great Lakes food web may be lessened, however the possibilities for ecosystem recovery remain unknown.

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Introduction:

Over 180 invasive species have become established in the Laurentian Great Lakes, leading to significant ecological and economic impacts that remain largely unpredictable (Vader Zanden et al, 2010). Of these invasions, the introduction and spread of both *Dreissena polymorpha* (zebra mussel) and *Dreissena rostriformis bugensis* (quagga mussel) in the late 1980's may be the most well-known. Both species have led to broad changes throughout the Great Lakes ecosystem since their introduction, particularly with their effects on nutrient cycling and alternations of food webs (Vanderploeg et al., 2002).

D. polymorpha is native to the Black and Caspian seas and was first reported in the Great Lakes in 1988; its mechanism of arrival was attributed to discharge of ballast water from ocean going vessels (Griffiths et al., 1991). *D. r. bugensis* is native to the Dnieper River drainage of Ukraine and was first discovered in the Great Lakes in 1989; however, it was not recognized as a separate species from *D. polymorpha* until 1991 (Mills et al., 1996).

Both *D. polymorpha* and *D. r. bugensis* are capable of filtering large volumes of water and reaching high population densities (Kryger and Riisgård, 1988; Horgan and Mills, 1997; Diggins, 2001). The large filtering capacity of both species has led to changes in both abiotic and biotic variables in the Great Lakes. These changes include increased water clarity, decreased concentrations of chlorophyll *a*, the loss of the spring phytoplankton bloom, and the sequestration of nutrients; these effects have been observed in both nearshore and offshore waters (Fahnenstiel et al., 1995; Hecky et al., 2004, Fahnenstiel et al., 2010, Vanderploeg et al., 2010). The spread of *Dreissena* has also been linked to the decline of the native amphipod *Diporeia* which served as an important link between upper and lower trophic levels in the Great

Lakes (Nalepa et al., 2009). Also, dreissenid mussels have been linked to increases in the nuisance benthic macro-algae *Cladophora* in lakes Erie, Michigan and Ontario (Auer et al., 2010).

D. polymorpha has mostly been replaced by *D. r. bugensis* throughout the Great Lakes (Wilson et al., 2006; Nalepa et al., 2009) and the latter is now the dominant dreissenid species in the lower Great Lakes. While *D. polymorpha* remains dominant in Lake Superior (Grigorovich et al., 2008), both species are limited in Lake Superior due to its physical and chemical characteristics (Grigorovich et al., 2003). Although *D. polymorpha* colonized the southern basin of Lake Michigan prior to *D. r. bugensis*, density and biomass of *D. polymorpha* began to decline after 2003, shortly after the appearance and expansion of *D. r. bugensis* (Nalepa et al., 2010). *D. r. bugensis* began to colonize the southern basin of Lake Michigan in 2000 and has been expanding from shallower sites to deeper regions since (Nalepa et al., 2001; Nalepa et al., 2010).

The displacement of *D. polymorpha* and subsequent expansion of *D. r. bugensis* has occurred due to several morphological and physiological differences between the species. Diggins (2001) showed that *D. r. bugensis* was able to filter up to 37% faster than *D. polymorpha,* with differences between the species becoming most apparent when individuals were exposed to warmer temperatures (14 °C and 22°C treatments). Aside from having a higher filtration rate, when food availability is low (seston ~2 $mg \cdot L^{-1}$), *D. r. bugensis* has been shown to have an assimilation efficiency of 81% as compared to an efficiency of 63% for *D. polymorpha* (Baldwin et al., 2002). *D. r. bugensis* is also able to put more of its energy into growth as it has a lower respiration rate than *D. polymorpha* (Stoeckmann, 2003).

Another unique and advantageous trait of *D. r. bugensis* in the Great Lakes is the presence of two distinct morphotypes, the eplimnetic morphotype and the profunda morphotype. The profunda morphotype has an elongated shell, is dorso-ventrally flattened, and is more pointed on its ventral end (Dermott and Munawar, 1993). Peyer et al. (2010) found that these two morphotypes are a result of phenotypic plasticity with temperature having the greatest effect on shell morphology. It is hypothesized that the presence of two distinct shell morphologies may play a role in the ability of *D. r. bugensis* to occupy a wider variety of habitats than *D. polymorpha* (Peyer et al., 2011). Aside from morphological differences, it was originally hypothesized that the profunda morphotype may have a longer inhalant siphon that may be an adaptation to feeding in deep water regions (Dermott and Munawar, 1993). Nalepa et al. (2013) found that there was considerable variation in ratios of siphon length to shell length in profunda morphotypes in Lake Michigan and in some cases these ratios were not significantly different from the shallow morph in Lake Erie. This variability and apparent phenotypic plasticity in siphon length likely allows *D. r. bugensis* to better adapt to environmental conditions.

Although *D. polymorpha* is one of the most studied freshwater invertebrates, much less information is known about *D. r. bugensis*. Since 1989 only 13% of all published papers relating to *Dreissena* are on *D. r. bugensis* (Karatayev et al., 2014). This lack of information on *D. r. bugensis* severely limits our ability to predict population growth and distributions, hence its future ecological impacts throughout the Great Lakes and other regions are unknown. While several studies have examined the population structure, nutritional state, and reproduction of *D. r. bugensis* at shallower sites, currently little is known about the nutritional state and

reproduction of *D. r. bugensis* in deep, consistently cold regions of the Great Lakes where the profunda morph dominates. Roe and MacIsaac (1997) published one of the first papers examining the reproductive status of *D. r. bugensis* in deep environments in Lake Erie and found evidence of gonadal development and spawning; however, their deepest site was only 55 m. In shallow waters spawning in *D. polymorpha* is initiated by increases in temperature (Bocherding, 1991); however, seasonal variations in temperature did not initiate spawning in individuals collected from deeper waters (>25 m) (Bacchetta et al., 2010). In deeper waters where temperatures are low throughout the year individuals likely rely on some other cue to initiate spawning (Kashian and Ram, 2013). Ram and Nichols (1993) proposed that this cue may come from chemicals associated with phytoplankton, although this remains unclear.

Since little is known about the thermal limits of *D. r. bugensis* there is uncertainty surrounding the recruitment and reproduction in deep offshore regions of the Great Lakes (Roe and MacIsaac 1997). *D. r. bugensis* recruits may be from local adults or from adults at nearshore sites (Martel et al., 2001). Information on the population structure and recruitment of *D. r. bugensis* in deeper regions of the Great Lakes is vital to aid in our understanding of this species.

The goals of this study are to: (1) Better understand population dynamics, nutritional state, and reproduction of *D. r. bugensis* in deeper regions of Lake Michigan as compared to shallower regions. (2) Determine if *D. r. bugensis* recruits in deeper offshore regions are from local individuals or are transported from nearshore sites. (3) Gain insight into possible future growth and expansion in deeper regions of Lake Michigan.

Methods:

Study Site:

Mussels were collected along a depth transect located in southern Lake Michigan off Muskegon, MI that has been part of NOAA's long term benthic monitoring program (Figure 1). The transect included a 25 m nearshore site (M-25; 43°12'00.0"N, 86°22'40.2"W), a 45 m intermediate site (M-45; 43°11'25.8"N, 86°25'43.2"W), and a 93 m offshore site (X-2; 43°12'00.0"N, 86°31'00.0"W). At each site mussels were collected monthly from April to September for use in computing length-weight regressions and to evaluate nutritional and reproductive state. Samples were also collected in spring, summer, and fall to evaluate trends in density and biomass at each site. Measurements for veligers and chlorophyll were taken at 15 m (M-15; 43°11'16.8"N, 86°20'38.4"W) and 110 m (M-110; 43°11'16.8"N, 86°32'09.6"W), as well as at M-45.

Temperature and Chlorophyll

Water temperatures were measured approximately 1 m above bottom using a SeaBird CTD on six dates that approximate mussel sampling dates (April 16th, May 13th, June 24th, July 24th, August 20th, and September 17th) in 2013. Measurements were taken at M-25, M-45, and X-2 on all dates except for April 16th and August 20th where measurements were taken at M-15 and M-110 in place of M-25 and X-2, respectively.

Water samples for chlorophyll were collected at M-15, M-45, and M-110 on the following dates: January 15th, March 14th, April 16th, May 9th, May 28th, Jun 17th, June 25th, July 8th, July 22nd, August 6th, August 20th, September 17th, September 27th, October 29th, and November 21st. On each date samples were taken with a modified Niskin bottle (Fahnenstiel et

al., 2002), at 5 m, 25 m, and 80 m at each of the three sites, respectively. Water was placed into acid-cleaned carboys, transported to the lab and placed into incubators at the appropriate temperature. Samples were filtered within 2 hours under low vacuum onto Whatman GF/F filters, and frozen at -80 °C until chlorophyll was analyzed. Filters were extracted with N, N–dimethylformamide (Speziale et al., 1984) and analyzed with a 10AU Turner fluorometer.

Density and Biomass:

Density and biomass of dreissenids were determined from samples taken at M-25, M-45, and X-2 in the spring, summer, and fall (March 29th, July 9th, and October 28th, respectively) in 2013. On each date triplicate Ponar grab samples were taken at each site, elutriated through a 500 µm mesh screen, and preserved in 5% formalin. All Dreissenids were counted under a dissecting microscope. Individuals greater than 5 mm were placed into vials and preserved in ethanol for biomass estimation, while individuals less than 5 mm were only counted. Seasonal density at each site is reported as a mean of the triplicate Ponar grab samples taken on each date.

Population biomass (as ash-free dry weight) was estimated by applying the length-weight regression relationship given by the equation $log_e AFDW\ (mg) = b + a log_e SL\ (mm)$, where AFDW=ash-free dry weight and SL= shell length. Length-weight relationships were developed on six dates (April 15th, May 13th, June 24th, July 24th, August 14th, and September 17th) in 2013. Additional measurements were also obtained during this process to calculate indices of nutritional state. On each of these sampling dates mussels were collected with Ponar grabs or a benthic sled, rinsed with lake water, and placed into coolers. Moist paper towels were placed over the mussels and cold packs were added to keep the mussels wet and cool

during transport to the laboratory. Within 48 hours of their collection live mussels were separated into five size classes based on shell length (10-12, 13-15, 16-18, 19-20, and ≥ 21 mm; classified as size class numbers 1, 2, 3, 4, and 5, respectively). Five individuals from each size class with a fully intact and undamaged shell were randomly selected. Shells were cleaned of all debris using a small razor blade, blotted dry, and weighed to obtain a whole wet weight. Shells were then opened and all soft tissue was removed and placed into pre-weighed aluminum planchets. Soft tissue was then dried at 60 °C for at least 48 hours. Each planchet was then reweighed so that shell-free dry weight (DW) could be obtained. Planchets containing dry soft tissue were then ashed for one hour at 550 °C and reweighed to obtain AFDW. Each shell was dried at room temperature for at least 48 hours before length and weight were measured. On some sampling dates a representative number of individuals in all five size classes were not present at some sites. This was a common occurrence at the 93 m site where larger individuals were often rare. In these cases extra mussels from the nearest size class were used to bring the total number of mussels examined at each depth to 25 for each sampling date.

Shell lengths of dreissenids over 5 mm in length collected on March 29th, July 9th, and October 28th were measured to be used in the length-weight regressions for biomass estimation using a scanner and IDL software developed at NOAA GLERL (Nalepa, unpublished). In most cases all dreissenids were measured; however, samples with more than 400 individuals were split by placing all individuals in a gridded tray and selecting random quadrates to sample at least 200 individuals. Mussels were then placed into 1-mm size categories ranging from 5 mm to 30 mm. AFDW of mussels in each size category were calculated using the appropriate length-weight regressions at the midpoint of that size category. Weight of all mussels less than

5 mm was calculated using the appropriate length-weight regression at a length of 3 mm. These weights were then multiplied by the total number of individuals in each size category and all weights were summed to give population biomass. Since length-weight regressions were not obtained in March or October, length-weight regressions from April and September were used to calculate biomass for those two months, respectively. Seasonal biomass for each site is reported as a mean of the triplicate Ponar grab samples taken on each date.

Nutritional State:

The nutritional state of mussels at M-25, M-45, and X-2 was determined from the same sets of mussels from which the length-weight regressions were developed. Nutritional state was determined using a condition index (CI) that estimates the amount of soft tissue within the given internal shell capacity. CI was calculated using the formula $CI = \frac{dry\ soft\ tissue\ weight\ (g) * 1000}{internal\ shell\ capacity}$ (Hawkins and Rowell, 1987; Crosby and Gale, 1990; Nalepa et al., 2010), where $internal\ shell\ capacity = whole\ wet\ weight - dry\ shell\ weight$ (Crosby and Gale, 1990). Since CI calculations utilize internal shell capacity, CI is particularly useful in cases when making comparisons between bivalve species that have different shell dimensions and thus these measurements could be useful in future studies involving different species (Nalepa et al., 2010). Ratios of shell weight to shell length (SW/SL), percent AFDW relative to DW, and percent of DW relative to wet weight (WW) were also calculated based on these measurements to aid in analysis of nutritional state. Length-weight regressions were also used as one index of nutritional state by calculating and comparing the weight of a standard 15-mm individual over time and at different locations (Nalepa et al., 1993, 1995, 2010).

Reproductive Trends:

Reproductive trends were examined on the same sampling dates as nutritional state in 2013. Within 48 hours of collection the gonads of ten mussels exceeding 20 mm in shell length from each depth were removed. This tissue was then placed onto a microscope slide, gently squashed with a cover slip and examined at various magnifications (up to 50X) under a compound microscope. Reproductive maturity was then categorized into one of five stages using a gametogenic index that was developed for monitoring sexual maturity in *D. polymorpha* (Table 1; Nichols, 1993).

Vertical net tows (153 µm net size opening) were used to collect dreissenid veligers on a monthly basis from January to December at M-15, M-45, and M-110 in 2013. In the laboratory, the samples were subsampled using a Hensen Stemple pipette and veligers were counted. The number of veligers was multiplied by mean DW according to Sprung (1984) to determine biomass.

Statistics

One way ANOVA was used to examine differences in densities and biomass between sites. Count data for densities was transformed using a square-root transformation; biomass data was transformed using a natural log transformation. To determine if there were site differences in CI, ratios of shell weight/shell length, AFDW/DW, and DW/WW, the non-parametric Kruskal-Wallis one-way analysis of variance and pairwise Mann-Whitney U post hoc tests were used since distributions were non-normal even after transformation. The Bonferroni approach was used to control for Type I error across post hoc tests. All statistical analysis was completed using the R statistical package (R Core Team, 2013).

Results:

Temperature and Chlorophyll:

Mean near bottom water temperature at M-25 (M-15), M-45, and X-2 (M-110) was 6.8 °C, 5.0 °C, and 4.0 °C, respectively (Figure 2). Bottom temperatures at M-25/M-15 were highest throughout the sampling season and also showed the largest fluctuations in temperature; temperatures ranged from a low of 3.9 °C in mid-April to a high of 9.1°C in June. Bottom temperatures at both M-45 and X-2 were less variable. Temperatures at M-45 ranged from 2.4 °C in mid- April to a high of 5.9 °C in mid-September; temperatures at X-2 ranged from 2.5 °C in mid- April to a high of 4.5 °C in June.

Mean chlorophyll in 2013 was 2.97 µg/L, 1.14 µg/L, and 0.467 µg/L at M-15, M-45, and M-110, respectively (Figure 3). Chlorophyll levels were most variable at M-15 with the largest peak occurring on May 9; chlorophyll peaked in June at M-45. Chlorophyll levels remained low at M-110 throughout the season, with peaks only occurring during spring and fall mixing in mid-March and late October.

Density and Biomass:

Total mean density (per m^2) over all sampling dates was 8811, 8796, and 12152 at 25, 45, and 93 m, respectively (Table 2). There was no significant difference between total mean annual density at the three depths ($F_{[2,24]=}$ 1.562, P=0.23). There were no significant differences in mean seasonal densities at any of the three sites (P=0.174, 0.991, and 0.922 at 25, 45, and 93 m, respectively).

Density (per m^2) of mussels less than 5 mm in length averaged over all sampling dates was 2830, 674, and 8794 at 25, 45, and 93 m, respectively (Table 3 and Figure 4). While there

were no significant differences in total densities there was a significant difference between mean density of mussels less than 5 mm in length at the three depths ($F_{[2,24]=}$ 28.25, P<0.001). Post hoc comparisons indicated that mean density of mussels less than 5 mm in length was significantly higher at 93 m than at both 25 m and 45 m (P<0.001). There was no significant difference (P=0.0925) between densities at 45 m and 25 m. There was no significant difference between mean density of mussels over 5 mm in length at the three depths ($F_{[2,24]}$= 1.467, P=0.23).

Mean shell length averaged over the entire sampling period was significantly different at the three depths (Table 3; $F_{[2,24]}$= 81.02, P<0.001). Shell length was greatest at 45 m with a mean length of 13.6 mm, followed by 25 m (8.0 mm) and 93 m (4.8 mm). Post hoc comparisons indicated that all pairwise comparisons were significantly different (P<0.001).

Mean annual AFDW biomass (g/m^2) over the entire sampling period was 35.98, 53.65, and 8.22 at 25, 45, and 93 m, respectively (Table 4). There were no significant differences between mean annual biomass at the three depths ($F_{[2,24]}$= 3.08, P=0.0643). When excluding 25 m due to the high standard deviation (52.04) observed at this site and looking at differences only between 45 m and 93 m there was a significant difference in mean annual biomass ($F_{[1,16]}$= 11.85, P=0.003). There were no significant differences in seasonal biomass at any of the three depths (P=0.313, 0.922, and 0.680 at 25, 45, and 93 m, respectively).

Nutritional State:

In 2013 mean CI over all sampling dates was 56.57, 32.39, and 37.87 at 25, 45, and 93 m, respectively. There was a significant difference between mean CI at the three depths (χ^2=197.14, P<0.001). Post hoc comparisons indicated that mean CI was significantly higher at

25 m than both 45 m and 93 m (P<0.001); mean CI at 93 m was significantly higher than that at 45 m (P<0.001).

Temporal fluctuation in CI also varied with depth (Figure 5). CI at 25 m remained highest of all depths throughout the entire sampling season; CI ranged from a low of 47.44 in April to a high of 75.00 in June. Overall fluctuation of CI was highest at this depth with large increases observed from April to June followed by large decreases from June to September. CI at 45 m remained the lowest of all depths throughout the entire sampling season; CI ranged from a low of 26.55 in August to a high of 38.31 in April. CI at 93 m remained intermediate throughout the sampling season; CI ranged from a low of 33.08 in August to a high of 42.28 in April. Monthly CI at 45 m and 93 m was less variable than that at 25 m; both sites showed decreases from April to May, increases from May to June, and decreases again from June to August, followed by increases from August to September.

When examining CI between the size classes within each depth there was no significant difference at 25 m (Table 5, Figure 6; χ^2=43.14, P=0.39). There was a significant difference in CI between size classes at 45 m (χ^2=1.13, P<0.001) and at 93 m (χ^2=58.89, P=<0.001). At 93 m differences were significant between each size class (P<0.005) except between size classes 1-2 and 4-3 (P=0.73 and 0.53, respectively). Overall there was a trend of decreasing CI with increasing size class with differences becoming less pronounced within the larger size classes.

Mean shell weight per unit shell length (SW/SL) over all sampling dates was 8.45, 6.52, and 6.42 at 25, 45, and 93 m, respectively (Figure 7). There was a significant difference between mean annual SW/SL at the three depths (χ^2=36.65, P<0.001). Post hoc comparisons indicated

that mean SW/SL in 2013 was significantly higher at 25 m than both 45 m and 93 m ($P<0.001$). There was no significant difference between mean SW/SL at 45 m and 93 m ($P=0.19$).

Mean percent AFDW of DW over all sampling dates was 89.05%, 86.58%, and 88.58% at 25, 45, and 93 m, respectively (Figure 8). There was a significant difference between mean annual AFDW of DW at the three depths ($\chi^2=57.40$, $P<0.001$). Post hoc comparisons indicated that mean AFDW of DW was significantly lower at 45 m than both 25 m and 93 m ($P<0.001$). There was no significant difference between mean percent AFDW of DW at 25 m and 93 m ($P=0.29$).

Mean percent tissue DW of WW over all sampling dates was 5.66%, 3.24%, and 3.79% at 25, 45, and 93 m, respectively (Figure 9). There was a significant difference between mean annual tissue DW of WW at the three sites ($\chi^2=197.14$, $P<0.001$). Post hoc comparisons indicated that mean tissue DW of WW was significantly higher at 25 m than both 45 m and 93 m ($P<0.001$). Mean tissue DW of WW was significantly higher ($P<0.001$) at 93 m than that at 45 m.

Differences between depths were also apparent when the linear relationship between AFDW and shell length were compared (Table 6). AFDW-SL regression slopes were significantly different between the three depths (ANCOVA $F_{[2,445]}= 6.05$; $P<0.01$). The slope at 25 m (2.988) was higher than that at both 45 and 93 m; however, the slope at 45 m (2.645) was equal to that at 93 m. Intercepts were also significantly different ($P<0.01$); the intercept was -5.986, -5.627, and -5.408 at 25, 45, and 93 m, respectively. The AFDW of a standard 15 mm individual as determined from the regression of AFDW and shell length also varied with depth (Table 6). AFDW of a 15 mm individual was 8.22, 4.64, and 5.78 at 25, 45, and 93 m, respectively.

Reproductive Trends:

Roughly half of all individuals at 25 m were mature (stage 4) on the first sampling date in April; all individuals had matured by July (Table 7). Half of all individuals at 45 m were mature in April; however patterns at this depth were more variable. All individuals at 93 m were mature during the first sampling in mid-April. Spawning began first at 45 m; spent (stage 5) mussels were found throughout the sampling season. Few spent mussels were found in April and May at 45 m; more than half were spent in July. Spawning at 45 m appeared to be complete by August as only one mature mussel was found after this date. Spawning began in August at 93 m and continued into September where 60% of individuals were spent while the remainder had not yet spawned. Spawning appears to have begun last at 25 m but was not completely captured in our surveys due to our inability to sample in October and November.

Peaks in veliger biomass were much higher at M-15 than those at either M-45 and M-110; the largest peak at M-15 was 0.3656 mg/m^3 in July whereas the largest peaks at M-45 and M-110 were 0.0488 mg/m^3 and 0.0521 mg/m^3, respectively, in early December (Figure 10). Veliger biomass first peaked in June at M-15 and M-45 while biomass at M-110 remained low. Another distinct seasonal peak occurred in late July (M-15) and early August (M-45 and M-110). A third seasonal peak occurred in September (M-15 and M-45) and October (M-110). Veliger biomass at all sites were similar and slightly elevated in mid-January and then began to decline after this sampling date; veligers were absent from mid-March until late-May at all sites.

Discussion:

Of all three sites, nutritional state of individuals at 25 m was highest based on all indices examined. CI at this site remained highest throughout the entire sampling period as did the

percentage of tissue DW relative to WW. Mean percentage of tissue AFDW relative to DW was also highest at 25 m. Results of the ANCOVA showed that the slope of the AFDW-SL regression was significantly highest at this site; AFDW of a 15 mm individual was also highest here. Although indices of nutritional state were highest at 25 m, biomass and average shell length were lower than at 45 m even though densities were similar.

The higher nutritional state but lower biomass and smaller average shell length of individuals at 25 m can be explained by a number of factors. Individuals at shallower depths must deal with frequent wave action, which leads to unstable substrates; this has been shown to decrease growth rates in *D. polymorpha* (Karatayev et al., 2006). This effect likely holds true for *D. r. bugensis* and could explain the smaller average size of individuals at this depth. Mussel distributions at nearshore sites are extremely patchy due to the relatively unstable environmental conditions at these sites (Karatayev et al., 2014; Nalepa et al., 2010). This patchiness can be seen when looking at standard errors of both densities and biomass at this site; this high variance at 25 m contributed to the result of non-significant differences in densities and biomass between sites. These unstable environmental conditions and patchiness could contribute to less overall competition between individuals. In other words the environmental conditions at this site may not be beneficial to long term growth at the site; however, they may have created ideal conditions for individuals that were present at the time of sampling (Nalepa et al., 2010), thus leading to a higher CI. In terms of other environmental conditions, chlorophyll levels were highest at this site throughout the majority of the sampling season, indicating that there was an abundant supply of phytoplankton in the water column for

mussels to feed on. The amount of chlorophyll per gram of AFDW biomass was 0.08 µg/L, the highest of all sites.

The relative nutritional state of mussels at 45 m and 93 m varied depending on the particular index. Interestingly CI at 93 m was significantly higher than that at 45 m; however, the results of the ANCOVA show that the slopes for the relationship between AFDW and shell length were the same. Measurements of percent AFDW of DW and percent DW of WW were significantly higher at 93 m. Differences in CI but matching slopes for increases in AFDW may be a result of the size of larval settlement. Martel et al. (2001) found that *D. r. bugensis* in Lake Erie settled at a larger size in offshore waters as compared to nearshore waters. If the pattern holds true between 45 m and 93 m sites it may indicate that individuals at 93 m were able to reach a larger size than those at 45 m early in their development, even though slopes of AFDW-SL regressions remain the same. Results support this as the intercept at 93 m (-5.408) was higher than at 45 m (-5.627), thus initial AFDW was higher at 93 m than at 45 m.

The lower CI at 45 m was likely a result of the high biomass that was found at this site; biomass at 45 m was 53.65 g AFDW/m^2, more than 6 times higher than the biomass found at 93 m. There were also a large number of larger individuals at 45 m; 20% of mussels found at this site were larger than 20 mm in length and average shell length was highest at this site at 13.6 mm. A higher biomass at 45 m indicates that there was higher intraspecific competition for food at this depth. The amount of chlorophyll per gram of AFDW biomass was 0.02 µg/L and 0.06 µg/L at 45 m and 93 m, respectively, thus there was more competition for food at 45 m than at 93 m.

Higher biomass at 45 m as compared to 25 m was likely a result of the relatively stable environmental conditions at this site that led to less overall mortality and allowed for long term growth. Temperature and chlorophyll measurements were more stable at this site throughout the sampling season, as was CI. Results of this study show that the shell weight per unit shell length (SW/SL) was significantly lower at 45 m as compared to 25 m. This shows that mussels at 45 m allocate less energy to shell growth, thus allowing more energy to be put towards tissue growth. These results correspond with previous studies that examine benthic biomass in Lake Michigan before the introduction of *D. polymorpha* and *D. r. bugensis* as biomass was greatest at intermediate depths (Nalepa, 1989). Nalepa et al. (2013) also found that *D. r. bugensis* has a significantly longer siphon length at 45 m as compared to individuals at 25 m and 93 m. This longer siphon may play a role in negating some of the effects of intraspecific competition at this site, thus allowing biomass to remain high.

Biomass at 93 m was lowest although density at this site was highest. This site was dominated by new recruits that were less than 5 mm in length; this size class represented 72% of the population at this depth. Hebert et al. (1990) found similar results in *D. polymorpha* population dynamics where the smallest shell sizes were found at sites that had the highest densities. Although environmental conditions are likely most stable here, food availability based on chlorophyll measurements was consistently low throughout the season as was temperature, both of which may contribute to slow growth and a low ultimate population biomass. Karatayev et al. (2011) found that *D. r. bugensis* grew more in warmer treatments (4–25 °C) than in cooler treatments (5-8 °C), thus growth rates may remain low at 93 m.

When examining CI of the various size classes, there were differences between sites. At both 45 m and 93 m, CI decreased as size class increased and differences between size classes were significant. A decrease in CI with increasing size may be an indication of limited food availability. Wacker and von Elert (2008) found that adult *D. polymorpha* required more than four times more food than juveniles to maintain zero growth. Although food availability might have been sufficient to maintain a high CI in smaller size classes, growth of larger individuals may have been inhibited by food availability at these sites. At 25 m there was only a slight decrease in CI between individuals from size classes 2 to 4 (13-15 mm to 19-21 mm) and these differences were not significant. These results show that food availability at this site was adequate for growth as even the largest mussels were able to maintain a high CI.

Patterns in monthly reproductive status varied considerably between the three sites. Individuals at 93 m and 45 m matured earliest as the majority of individuals sampled were gravid during the first sampling event in April. Individuals at 25 m did not mature until later in the season; 70% of individuals sampled were mature by June. It is difficult to say why individuals matured later at 25 m; however, it may be related to the unstable environmental conditions and rapid increase in temperature at this nearshore site.

Results of spawning at 25 m and 45 m are consistent with the temporal trends observed in 2008 (Nalepa et al., 2010), although spawning seems to have been slightly delayed at both sites in 2013. Mean bottom temperature was 10 °C in 2008, while the mean bottom temperature in 2013 was only 6.8 °C; this difference in temperature could explain the delay in spawning at 25 m. Differences in temperature could not explain the delay in spawning at 45 m as they were similar throughout both years. Spawning at 45 m and 93 m started when

temperatures began to stabilize after the initial warming period in early spring, and were in the range of 5-6 °C and 4-5 °C, respectively. Roe and MacIsaac (1997) also found spent individuals in Lake Erie at 37 m and 55 m sites when temperatures were in the range of 4.8-6 °C. These results indicate that spawning in *D. r. bugensis* may be triggered by a temperature increase; however, spawning at 25 m did not support this idea. If an increase in temperature was the trigger to spawning we should have observed spent individuals at 25 m first, but this was not the case. Spawning in *D. polymorpha* has a minimum temperature threshold, and an increase in temperature along with temperature stability may provide ideal conditions for spawning but not directly trigger the spawning event (Ram and Nichols, 1993). It is possible that the increase in temperature in combination with temperature stability may be the final cue needed for spawning at offshore sites, although this increase from early spring temperatures was minimal. Large fluctuations in temperatures at 25 m may have delayed spawning until later in the season. Environmental cues that trigger spawning in *D. r. bugensis* remain unknown. Spawning has been induced in laboratory experiments using serotonin, temperature shock, and the freshwater alga *Chlorella minutissima* but limited field testing has been performed (Kashian and Ram, 2013; Schwaebe et al., 2013)

Peaks in veliger biomass at 45 m and 110 m matched well with spawning events at the 45 m and 93 m sites, respectively, with peaks appearing in mid-June and August. As Nalepa et al. (2010) observed in 2008 there were also slight peaks in veliger biomass at one depth when spawning at another depth took place. There were several peaks in veliger biomass at 15 m which occurred before our last sampling for reproductive status in September when individuals at 25 m were still gravid. A large peak in veliger biomass occurred in July at 15 m and was more

than 5 times higher than the largest peaks at 45 m and 110 m indicating that veligers present at this location were likely coming from spawned adults at other locations. Peaks in veliger biomass at 93 m that correspond with observed spawning events, along with the presence of gravid females, indicate *that D. r. bugensis* does in fact spawn and local recruitment occurs at depths greater than 90 m in Lake Michigan.

Although changes in tissue mass before and after spawning events have been used in the past to estimate reproductive effort in *D. polymorpha* (Nalepa et al. 1993; Chase and Bailey 1999) and *D. r. bugensis* (Nalepa et al., 2010), results for seasonal trends in tissue mass did not match as well with spawning patterns in 2013. CI dropped by 9% from June to July at 45 m; differing from the 26% decline observed after spawning by Nalepa et al. (2010). At 93 m there was only a 5% decline in CI from July to August when spawning began, although only about half of all individuals had spawned at this depth. Following this time period CI increased by 7% from August to September even though spawning was still occurring based on gametogenic indices. While these declines in CI may be in part due to losing tissue mass following spawning events it is impossible to rule out changes in food availability from these patterns. For example, there are several fluctuations in chlorophyll levels from June through July and CI dropped by 18% during the same time period, thus changes in food availability likely contributed to the decline in CI observed here. Since the spawning event at 25 m was not captured in this study environmental change should be the dominant driver explaining month to month variation in CI after maturation. Chlorophyll levels showed a large peak in mid-May, and subsequently this peak was followed by a 37% increase in CI in June. This peak was then followed by a 32% decrease in CI from July to August; chlorophyll levels dropped from 7.91 µg/L to 2.03 µg/L

during the same time period. These results show that caution should be used when attempting to show reproductive effort solely based upon changes in tissue mass.

Trends in density, biomass, and condition index:

Densities at all three sites were lower in 2013 than in 2010, the last time all three sites were sampled (Figure 11; data prior to 2013 provided by T. Nalepa, unpublished). At 45 m density peaked in 2007 and has been decreasing since, although density now appears to be stabilizing. Densities at both 25 m and 93 m appear to have peaked in 2010 and are now declining. The trends at these three sites match the overall patterns observed in southern Lake Michigan based upon results of a long-term benthic monitoring program that included 40 sites sampled each year between 1998 and 2010, except for 2009 when no survey was conducted (Nalepa et al., 2010; Nalepa et al., 2013).

In 2013 biomass at 25, 45, and 93 m was 36.0, 53.7, and 8.2 g/m^2, respectively, compared to 19.3, 52.7, and 7.5 g/m^2 in 2010 (Nalepa et al., 2013). At 45 m biomass has only increased by 2% from 2010 to 2013, which matches with the general trend of a stable density in the same time period at this depth. Biomass at 25 m has increased by 86% since 2010, but this increase does not correspond well with the overall decline in density at this depth. This inconsistency could be a result of an increase in average size of individuals. Individuals at 25 m had an average length of 8.0 mm while individuals at sites in the southern basin within the 16-30 m depth interval had an average length of 3.1 mm in 2008 (Nalepa et al., 2010). Results at such shallow depths are difficult to interpret due to the large fluctuations observed as a result of unstable environmental conditions. Biomass increased by 10% at 93 m from 2010 to 2013. Based upon comparisons between 2013 data at X-2 and data from the six Lake Michigan

southern basin sites from Nalepa et al. (2010) that are over 90 meters in depth it appears that the average size of mussels at this depth is slowly increasing. The proportion of mussels ranging from 10-15 mm has increased from 3.3% in 2008 to 7.0% in 2013 and the proportion of mussels ranging from 15-20 mm has increased from 0.2% to 2.3% during the same time period. While larger individuals are becoming more common at this depth the average size of individuals has only increased slightly; in 2008 the average size was 3.9 mm compared to an average size of 4.77 mm in 2013. This slight increase in overall size indicates that food availability and temperature were not limiting overall growth at this depth; however, as previously discussed the lower condition observed in larger individuals may suggest food was limiting for larger individuals. It is also apparent that this site was still in the early stages of colonization as 72% of individuals were less than 5 mm in length, which was a significantly higher proportion than the other two sites. Expansion at 45 m seems to have slowed, but the large numbers of young *D. r. bugensis* suggest that expansion could be occurring at greater depths, including areas greater than 90 m.

CI at 25 m and 45 m was lower than in previous years and thus consistent with a continued decline (Figure 12; 2004 and 2008 data from Nalepa et al, 2010). CI at 25 m decreased by 9% from 2004 to 2008 and decreased by 24% from 2008 to 2013. CI at 45 m decreased by 31% from 2004 to 2008 and decreased by 22% from 2008 to 2013. CI at 93 m was not measured in 2004 or 2008. Continued declines in CI at these sites are likely a result of two factors. Populations at these sites now have a greater proportion of larger individuals which require more food per individual (Wacker and von Elert, 2008), and less food is available since

summer chlorophyll *a* concentrations in southeastern Lake Michigan have declined since *D. r. bugensis* first began to colonize this region of Lake Michigan (Pothoven and Fahnenstiel, 2013).

Future work:

These results show evidence of continued population growth and reproduction in depths greater than 90 m, hence the ultimate size of the population relative to the carrying capacity at these depths remain unknown. Growth appears to be stabilizing at intermediate depths but continued monitoring is necessary to determine if this trend holds true or the lag in growth is only temporary. Continued research is needed to examine the exact mechanism between the large fluctuations that are observed in densities and abundances of *D. r. bugensis* in nearshore habitats. The exact environmental cues that trigger spawning in *D. r. bugensis* remain unclear and require further inquiry.

Continued monitoring of trends in condition, biomass, and reproduction of *D. r. bugensis* is needed to understand the numerous effects of their continued spread. These trends need to be taken into consideration when making both long and short term management decisions in Lake Michigan. If populations of *D. r. bugensis* follow a boom-and-bust cycle as *D. polymorpha* has there may be hope for ecosystem recovery, but if populations continue to fluctuate, adapting lake-wide management plans will become more difficult (Strayer and Malcom, 2006).

Tables and Figures

Table 1: Gametogenic index (Nichols, 1993) used to determine sexual maturity of *Dreissena polymorpha*

	Female	**Male**
Gametogenic Stage	**Features**	**Features**
Stage 1	Round cells; no nuclei or germinal vesicle present, unable to distinguish males from females	Round cells; no nuclei or germinal vesicle present, unable to distinguish males from females
Stage 2	Nuclei present, no germinal vesicle	Round cells, look like stage 1
Stage 3	< 50% of gametes have nuclei and germinal vesicle (>50% still at stage 2)	< 50% gametes triangular shape with tails (>50% still at stage 2)
Stage 4	> 50% of gametes have nuclei and germinal vesicle (<50% still at stage 2)	> 50% gametes triangular shape with tails (<50% still at stage 2)
Stage 5	Only a few stage 4 eggs remain, lots of empty spaces	Only a few stage 4 sperm remain, lots of empty spaces

Table 2: Mean Seasonal densities (±SE) per m^2 at M-25, M-45, and X-2.

	Station		
Date	M-25	M-45	X-2
29 March	17,600 ± 8,693	10,125 ± 4,829	11,902 ± 2,544
09 July	8,190 ± 2,612	7, 626 ± 2,388	12,923 ± 2,277
28 October	643 ± 75	8,639 ± 3,818	11,631 ± 1,967
Mean	8,811 ± 3,589	8,796 ± 1,940	12,152 ± 1,154

Table 3: Percentage of individuals in each size class and average length (mm) during each sampling date at M-25, M-45, and X-2

	Size Category (mm)						
Station/Date	0-5	5-10	10-15	20-25	20-25	>25	Mean Length
M-25							
29 March	26.52	26.12	29.15	14.80	3.00	0.41	9.7
09 July	54.89	19.06	16.18	7.69	2.05	0.12	6.8
28 October	53.12	16.42	11.34	14.76	4.37	0.00	7.6
Mean	44.84	20.53	18.89	12.42	3.14	0.18	8.0
M-45							
29 March	9.71	28.64	24.78	17.18	17.63	2.06	13.1
09 July	9.19	16.13	18.52	30.76	22.89	2.51	15.0
28 October	8.39	29.45	27.61	19.72	12.53	2.29	12.7
Mean	9.10	24.74	23.64	22.55	17.68	2.29	13.6
X-2							
29 March	76.76	14.03	6.85	2.03	0.26	0.06	4.5
09 July	69.50	20.38	7.31	2.34	0.47	0.00	4.9
28 October	70.37	19.95	6.74	2.37	0.31	0.25	4.8
Mean	72.21	18.12	6.97	2.25	0.35	0.10	4.8

Table 4: Mean seasonal AFDW biomass per m^2 (±SE) at M-25, M-45, and X-2.

	Station		
Date	M-25	M-45	X-2
29 March	83.42 ± 41.47	69.69 ± 35.39	7.18 ± 1.51
09 July	22.37 ± 10.01	50.55 ± 20.02	9.24 ± 1.62
28 October	2.16 ± 0.77	40.70 ± 22.89	8.22 ± 1.50
Mean	35.98 ± 17.35	53.65 ± 14.13	8.22 ± 0.83

Table 5: Results of the post hoc comparisons of condition index at each size class using a pairwise Mann-Whitney U test. Size class 1, 2, 3, 4, and 5 correspond to mussels that are 10-12, 13-15, 16-18, 19-20, and ≥21 mm in shell length, respectively. There was no significant difference between size classes at M-25 so no post hoc comparison was performed.

Size Class Comparison	M-45	X-2
2v1	0.002	0.730
3v1	<0.001	<0.001
4v1	<0.001	<0.001
5v1	<0.001	<0.001
3v2	0.321	<0.001
4v2	0.009	<0.001
5v2	<0.001	<0.001
4v3	0.230	0.533
5v3	0.024	0.001
5v4	0.155	0.005

Table 6: Regression coefficients for the relationship between shell length (SL) and tissue ash-free dry weight (AFDW) given by the equation $log_e AFDW\ (mg) = b + a log_e SL\ (mm)$. The ash-free dry weight (mg) of a 15-mm individual as determined by the relationship is also given.

Station/Date	b	a	R^2	n	15-mm
M-25					
15 April	-5.497	2.739	0.832	25	6.83
13 May	-5.920	2.965	0.942	25	8.25
24 June	-5.021	2.752	0.843	25	11.38
24 July	-6.632	3.247	0.880	25	8.67
14 August	-6.455	3.140	0.888	25	7.74
17 September	-6.014	2.953	0.935	25	7.25
Overall	-5.986	2.988	0.847	150	8.22
M-45					
15 April	-6.722	3.086	0.934	25	5.13
13 May	-5.444	2.588	0.964	25	4.78
24 June	-6.065	2.834	0.925	25	5.00
24 July	-5.212	2.500	0.823	25	4.75
14 August	-4.882	2.334	0.825	25	4.21
17 September	-5.836	2.692	0.956	25	4.28
Overall	-5.627	2.645	0.891	150	4.64
X-2					
15 April	-6.257	2.971	0.961	26	5.98
13 May	-5.610	2.744	0.965	25	6.18
24 June	-4.933	2.507	0.957	25	6.40
24 July	-5.526	2.677	0.970	25	5.60
14 August	-5.142	2.506	0.950	25	5.17
17 September	-5.630	2.714	0.977	25	5.58
Overall	-5.408	2.645	0.950	151	5.78

Table 7: Percentage of individuals at each stage of gametogenesis during each sampling date at M-25, M-45, and X-2.

Date/Stage	M-25					M-45					X-2				
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
15-Apr	21.4	0.0	14.3	57.1	7.1	0.0	14.3	14.3	50.0	21.4	0.0	0.0	0.0	100.0	0.0
13-May	15.4	7.7	30.8	46.2	0.0	50.0	0.0	7.1	35.7	7.1	0.0	0.0	0.0	92.3	7.7
24-Jun	0.0	0.0	20.0	70.0	10.0	10.0	0.0	30.0	40.0	20.0	0.0	0.0	0.0	100.0	0.0
24-Jul	0.0	0.0	0.0	100.0	0.0	0.0	0.0	10.0	30.0	60.0	0.0	0.0	0.0	90.0	10.0
14-Aug	0.0	0.0	0.0	100.0	0.0	40.0	0.0	0.0	0.0	60.0	0.0	0.0	0.0	54.5	45.5
17-Sep	0.0	0.0	0.0	90.0	10.0	40.0	0.0	0.0	10.0	50.0	0.0	0.0	0.0	40.0	60.0

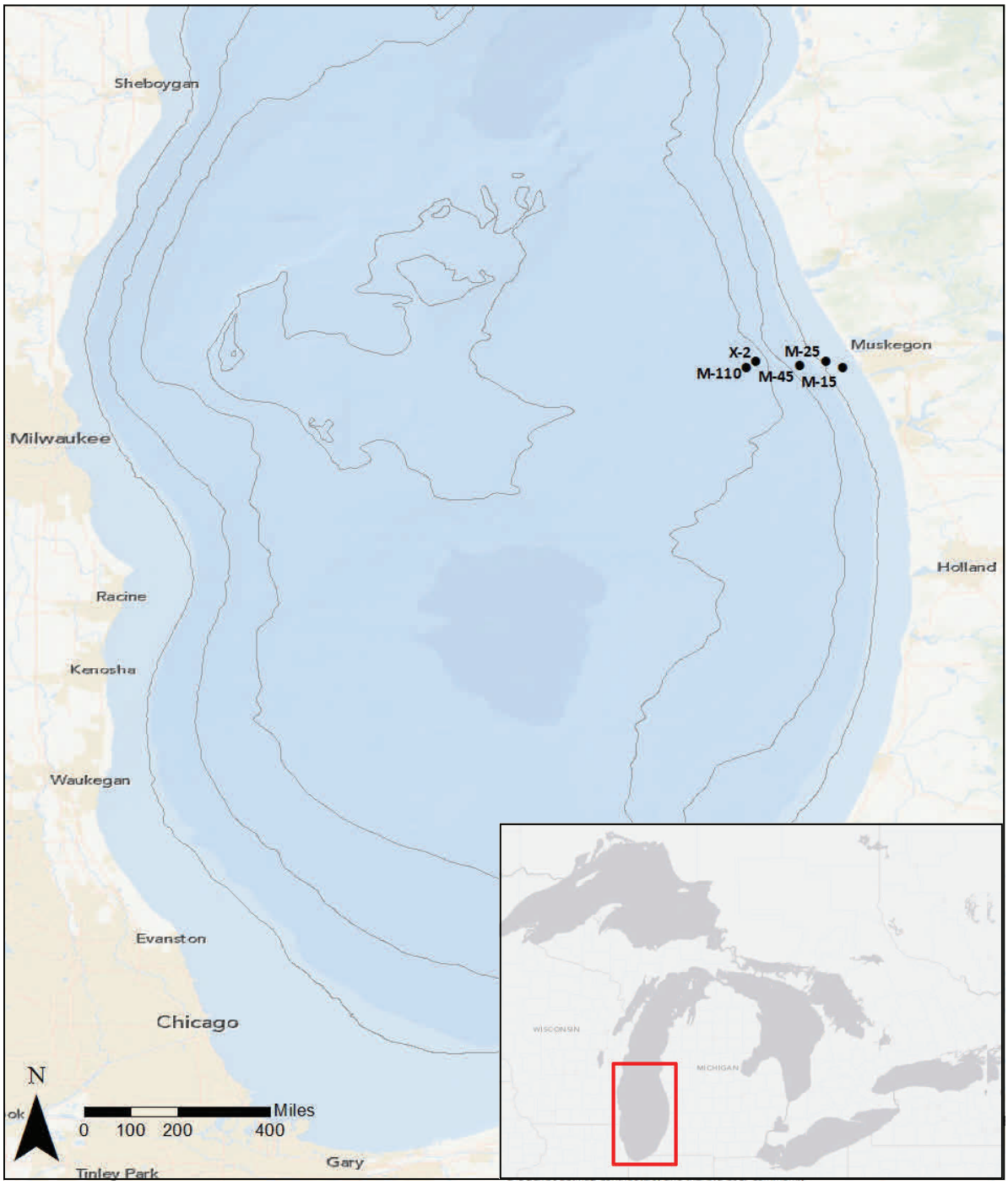

Figure 1: Location of M-15 (15 m), M-25 (25 m), M-45 (45 m), X-2 (93 m) and M-110 (110 m) sampling sites in southern Lake Michigan.

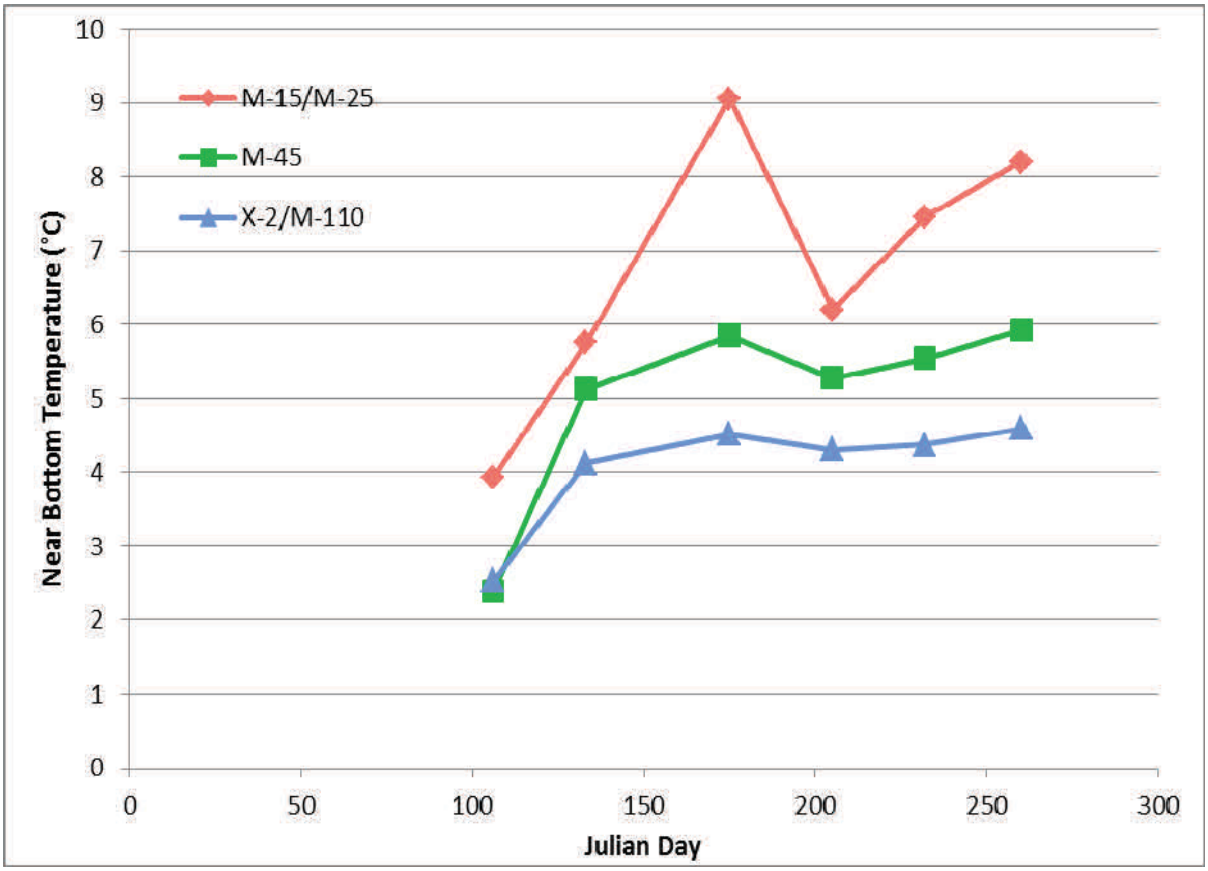

Figure 2: Near bottom temperature at M-15/M-25, M-45, and X-2/M-110.

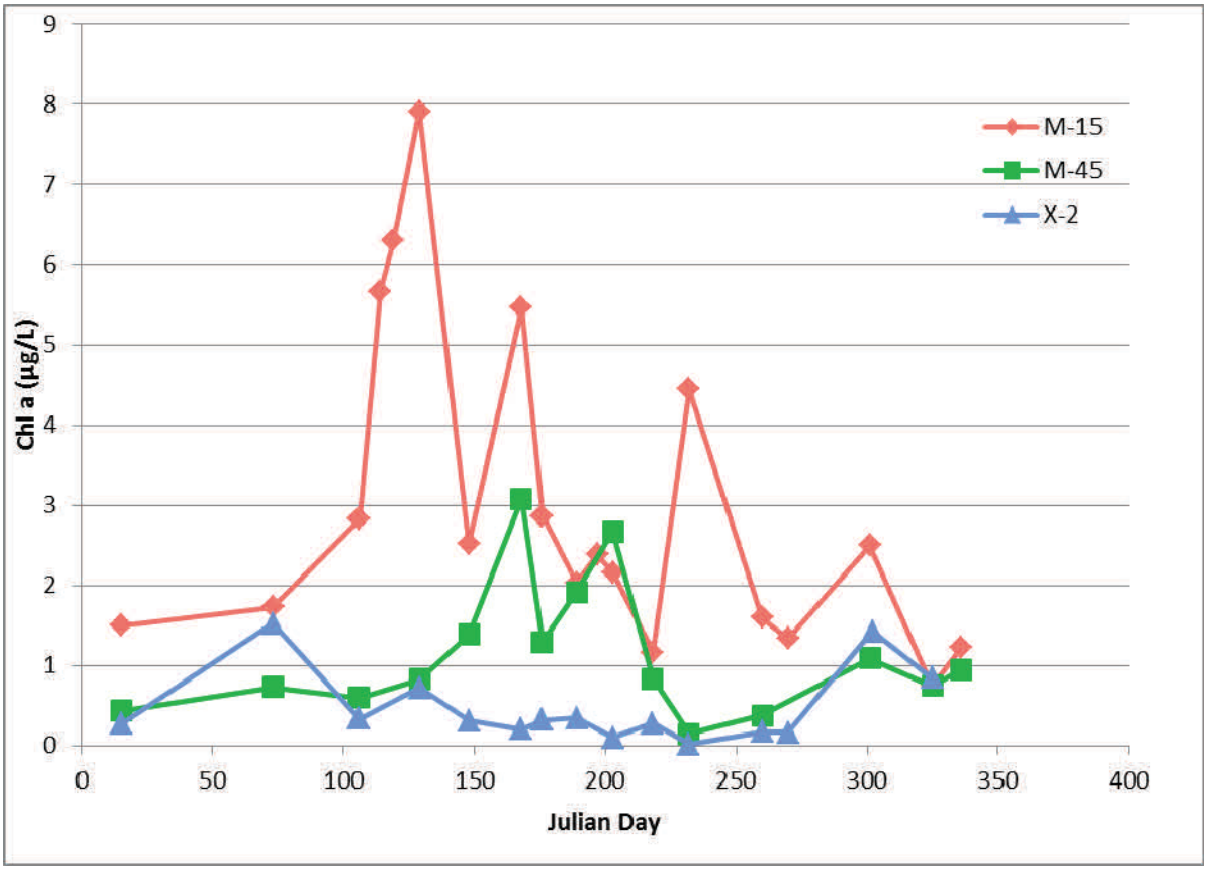

Figure 3: Chlorophyll measured at M-15, M-45, and M-110; measurements were taken at 5 m, 25 m, and 80 m, respectively.

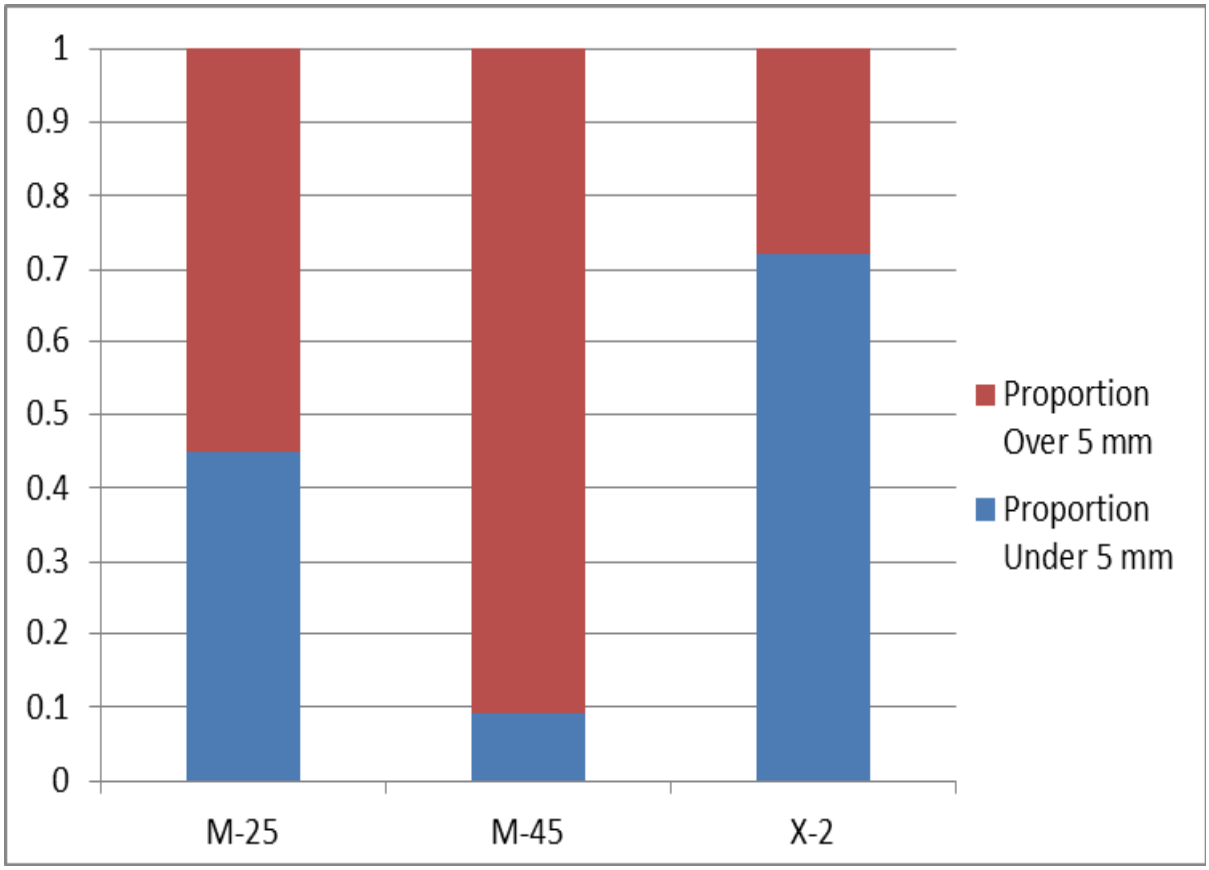

Figure 4: Mean proportion of mussels over and under 5 mm at M-25, M-45, and X-2

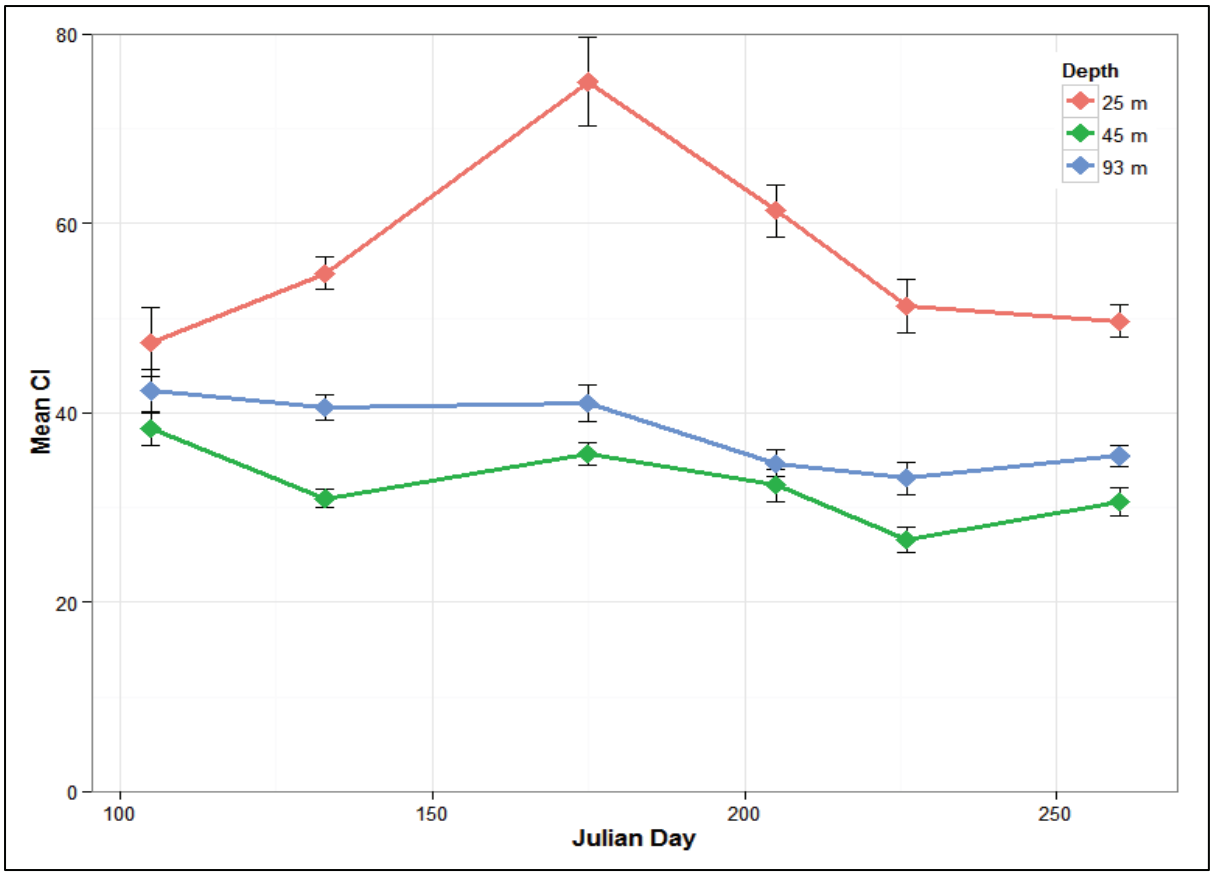

Figure 5: Mean (±SE) seasonal condition index from April to September 2013 at M-25, M-45, and X-2.

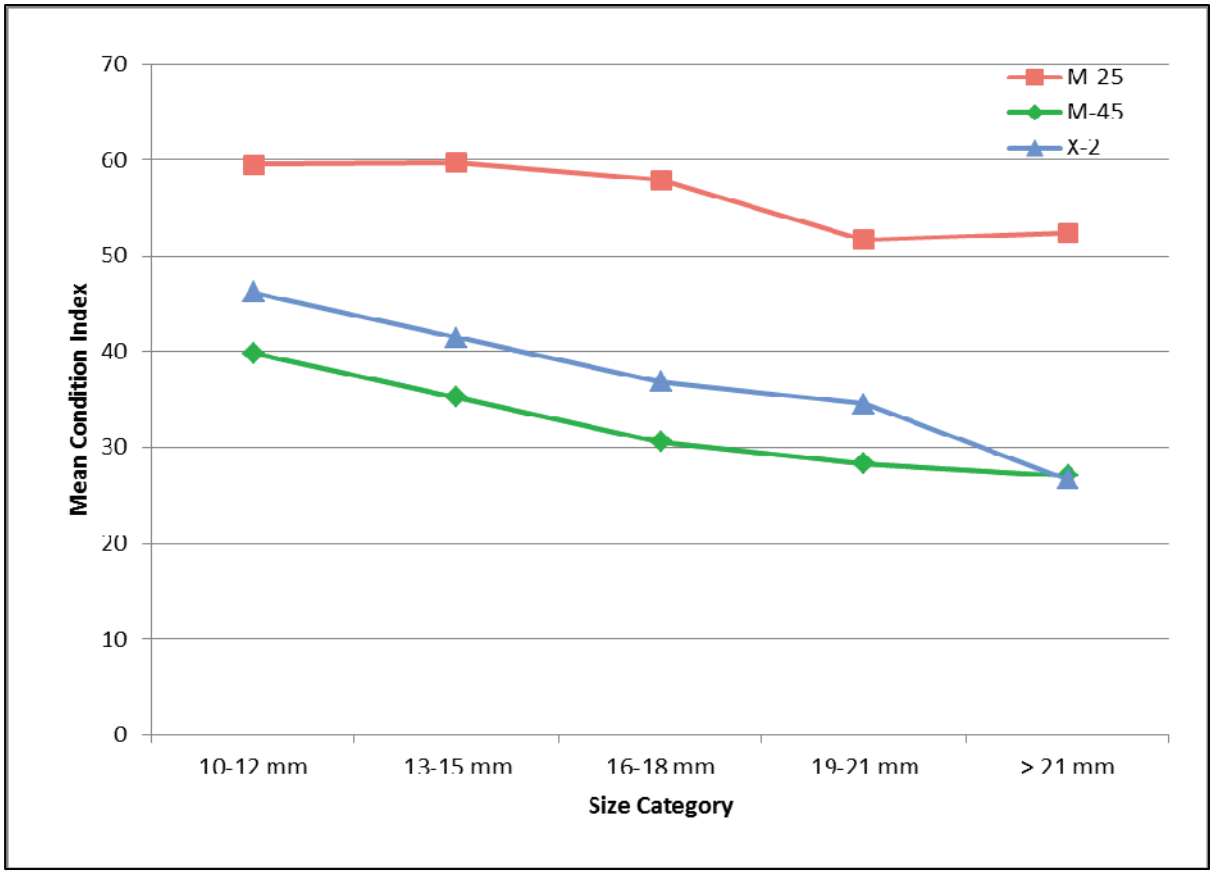

Figure 6: Mean condition index at of each size class at M-25, M-45, and X-2

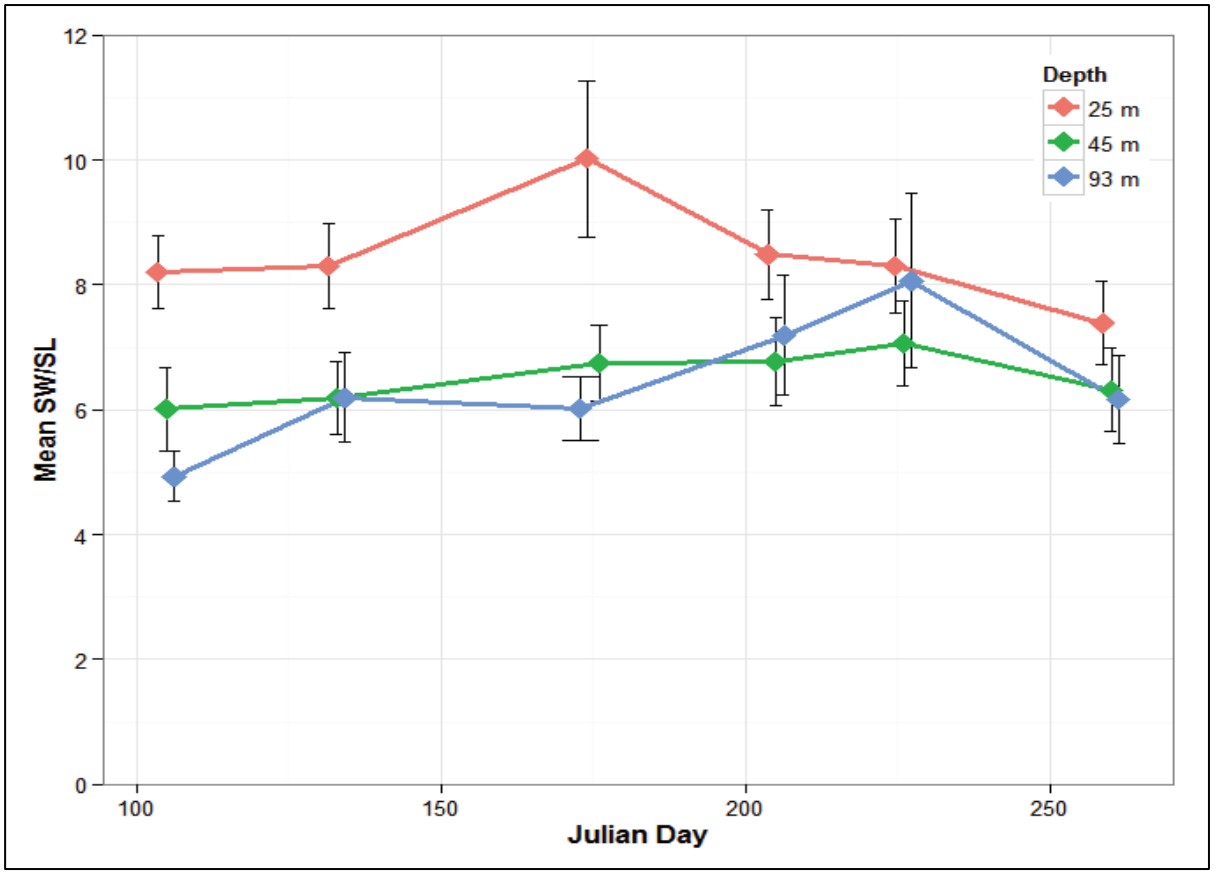

Figure 7: Mean seasonal shell weight per shell length (±SE) at M-25, M-45, and X-2.

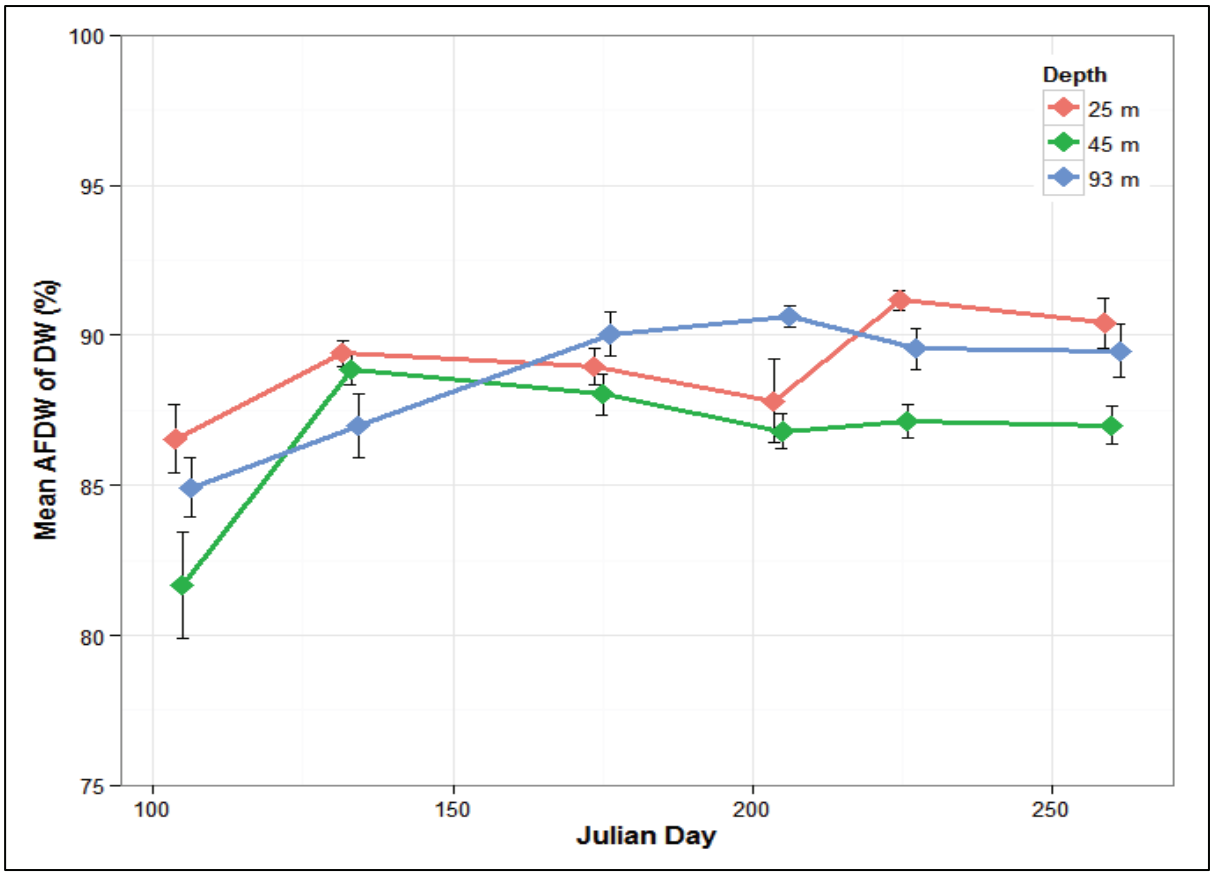

Figure 8: Mean seasonal AFDW of DW (%) (±SE) at M-25, M-45, and X-2.

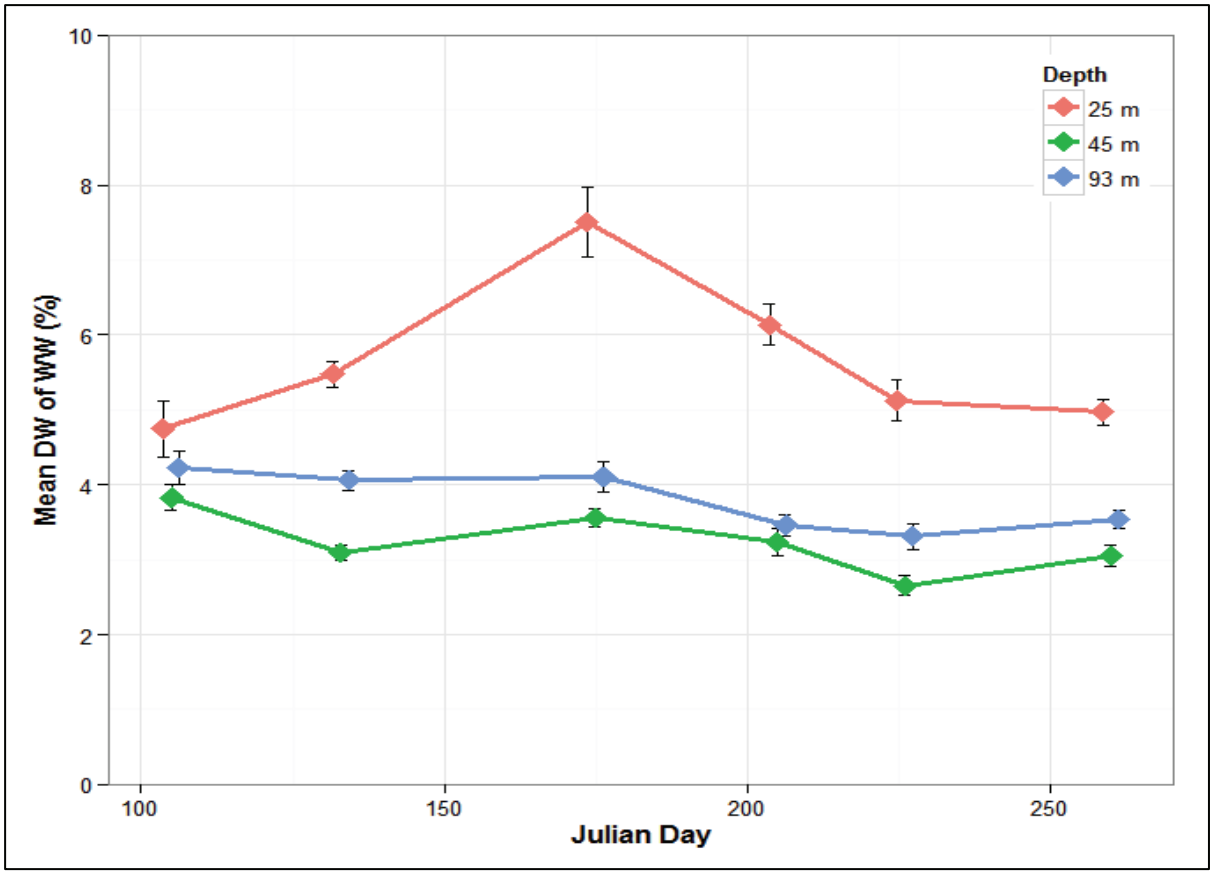

Figure 9: Mean seasonal dry weight of wet weight (%) (±SE) at M-25, M-45, and X-2.

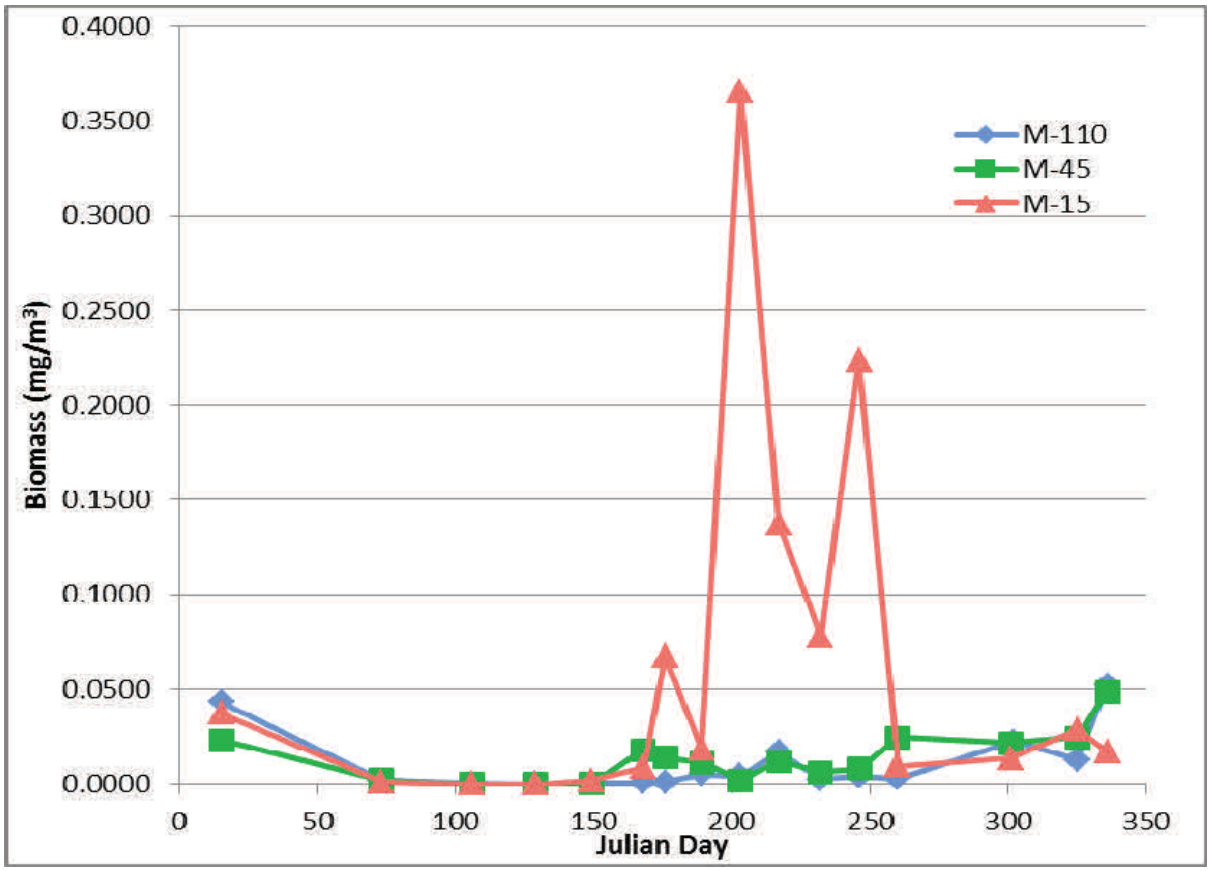

Figure 10: Veliger biomass (as dry weight) at M-15, M-45, and M-110.

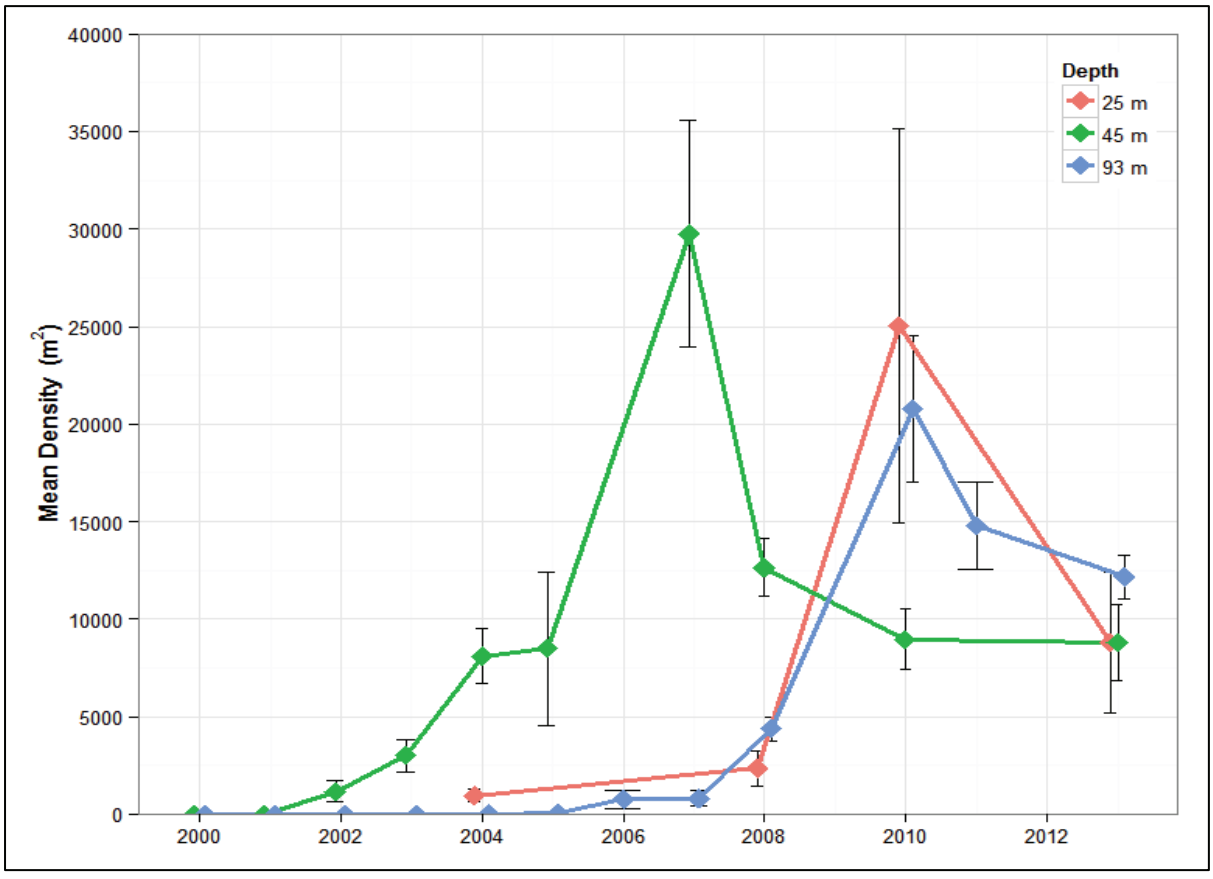

Figure 11: Trends in density (mean ±SE) at 25, 45, and 93 m. Data prior to 2013 provided by T. Nalepa (unpublished).

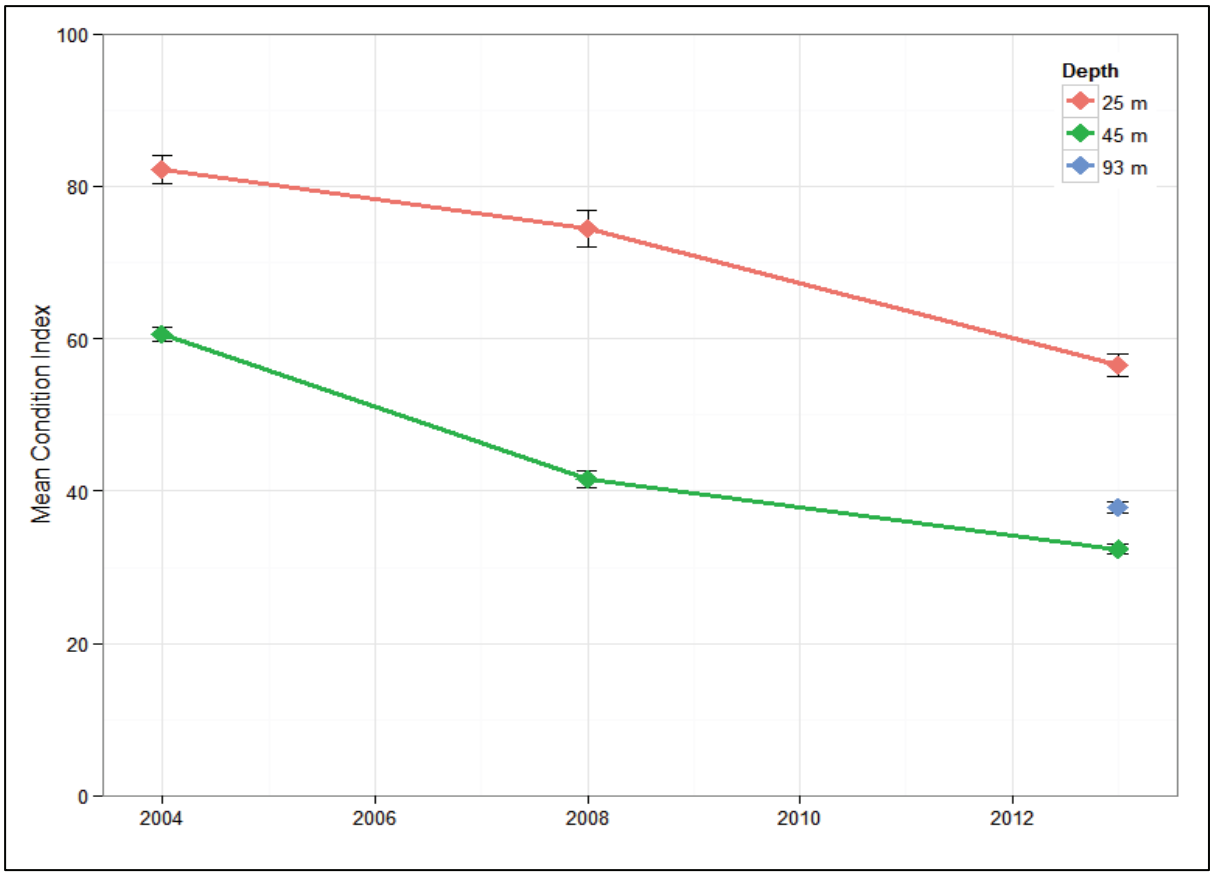

Figure 12: Trends in condition index (mean ±SE) since 2004 at 25, 45, and 93 m. 2004 and 2008 data from Nalepa et al. (2010); CI at 93 m was not examined in 2004 or 2008.

Literature Cited:

Auer, M.T., Tomlinson, L.M., Higgins, S.N., Malkin, S.Y., Howell, E.T., Bootsma, H. a., 2010. Great Lakes *Cladophora* in the 21st century: same algae—different ecosystem. J. Great Lakes Res. 36, 248–255.

Bacchetta, R., Mantecca, P., Vailati, G. 2010. Reproductive behavior of zebra mussels living in shallow and deep water in the South Alps lakes. In The Zebra Mussel in Europe. G. van der Velde, S. Rajagopal, and A. bij de Vaate (Eds.). Backhuys Publishers, Leiden, The Netherlands, pp. 161-168.

Baldwin, B.S., Mayer, M.S., Dayton, J., Pau, N., Mendilla, J., Sullivan, M., Moore, A., Ma, M., Mills, E.L., 2002. Comparative growth and feeding in zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*); implications for North American lakes. Can. J. Fish. Aquat. Sci. 59, 680–694.

Borcherding, J., 1991. The annual reproductive cycle of the freshwater mussel *Dreissena polymorpha* Pallas in lakes. Oecologia 87, 208–218.

Chase, M.E., Bailey, R.C., 1999. The ecology of the zebra mussel (*Dreissena polymorpha*) in the lower Great Lake of North America: II. Total production, energy allocation, and reproductive effort. J. Great Lakes Res. 25, 1122–1134.

Crosby, M.P., Gale, L.D., 1990. A review and evaluation of bivalve condition methodologies with a suggested standard method. J. Shellfish Res. 9, 233-237.

Dermott, R. and Munawar, M. (1993). Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. Can. J. Fish. Aquat. Sci. 50, 2298-2304.

Diggins, T.P., 2001. A seasonal comparison of suspended sediment filtration by quagga (*Driessena bugensis*) and zebra (*D. polymorpha*) mussels. J. Great Lakes Res. 27, 457–466.

Fahnenstiel, G.L., Lang, G.A., Nalepa, T.F., Johengen, T.H., 1995. Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. J. Great Lakes Res. 21, 435–448.

Fahnenstiel, G.L., Beckmann, C., Lohrenz, S.E., Millie, D.F., Schofield, O.M.E., McCormick, M.J., 2002. Standard Niskin and Van Dorn bottles inhibit phytoplankton photosynthesis in Lake Michigan. Verh. Int. Ver. Limnol. 28, 376–380.

Fahnenstiel, G., Pothoven, S., Nalepa, T., Vanderploeg, H., Klarer, D., Scavia, D., 2010. Recent changes in primary production in the offshore region of southeastern Lake Michigan. J. Great Lakes Res. 36 (Supplement 3), 20–29.

Griffiths, R.W., Schloesser, D.W., Leach, J.H., Kovalak, W.P., 1991. Distribution and Dispersal of the Zebra Mussel (*Dreissena polymorpha*) in the Great Lakes Region. Can. J. Fish. Aquat. Sci. 48, 1381–1388.

Grigorovich, I., Korniushin, A., Gray, D., 2003. Lake Superior: an invasion coldspot? Hydrobiologia 499, 191–210.

Grigorovich, I., Kelly, J., Darling, J., West, C., 2008. The quagga mussel invades the Lake Superior basin. J. Gt. Lakes Res. 34, 342–350.

Hawkins, C.M. and Rowell, T.W., 1987. The importance of cleansing in the calculation of condition index in the soft-shell clam, *Mya arenaria* (L.). J. Shellfish Res. 6, 85-88.

Hecky, R.E., Smith, R.E.H., Barton, D.R., Guildford, S.J., Taylor, W.D., Charlton, M.N., Howell, T., 2004. The nearshore phosphorus shunt: a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. Can. J. Fish. Aquat. Sci. 61, 1285–1293.

Hebert, P.D.N., Wilson, C.C., Murdoch, M.H., Lazer, R., 1991. Demography and ecological impacts of the invading mollusc *Dreissena polymorpha*. Can. J. Zool. 69, 405–409.

Horgan, M.J., Mills, E.L., 1997. Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): implications for freshwater lakes. Can. J. Fish. 54, 249–255.

Karatayev, A., Burlakova, L., Padilla, D., 2006. Growth rate and longevity of *Dreissena polymorpha* (Pallas): a review and recommendations for future study. J. Shellfish Res. 25, 23–32.

Karatayev, A., Mastitsky, S., Padilla, D., 2011. Differences in growth and survivorship of zebra and quagga mussels: size matters. Hydrobiologia 668, 183–194.

Karatayev, A., Burlakova, L., Padilla, D., 2014. Zebra versus quagga mussels: a review of their spread, population dynamics, and ecosystem impacts. Hydrobiologia (in press).

Kashian, D.R, Ram, J.L. Chemical regulation of Dreissenid reproduction. 2013. In Quagga and Zebra Mussels: Biology, Impacts, and Control, Second Edition. T.F. Nalepa, and D.W. Schlosser (Eds.). CRC Press, Boca Raton, FL, pp. 461-468

Kryger, J., and Riisgård, H.U. 1988. Filtration rate capacities in 6 species of European freshwater bivalves. Oecologia, 77: 34–38.

Martel, A.L., Baldwin, B.S., Dermott, R.M., Lutz, R.A., 2001. Species and epilimnion/ hypolimnion-related differences in size at larval settlement and metamorphosis in *Dreissena* (Bivalvia). Limnol. Oceanogr. 46, 707–713.

Mills, E.L., Rosenberg, G., Spidle, a P., Ludyanskiy, M., Pligin, Y., May, B., 1996. A review of the biology and ecology of the quagga mussel (*Dreissena bugensis*), a second species of freshwater dreissenid introduced to North America. Am. Zool. 36, 271–286.

Nalepa, T.F., 1989. Estimates of macroinvertebrate biomass in Lake Michigan. J. Great Lakes Res. 15, 437–443.

Nalepa, T.F., Cavaletto, J.F., Ford, M., Gordon, W.M., Wimmer, M., 1993. Seasonal and annual variation in weight and biochemical content of the zebra mussel, *Dreissena polymorpha*, in Lake St. Clair. J. Great Lakes Res 19, 541–552.

Nalepa, T.F., Wojcik, J.A., Fanslow, D.L., Lang, G.A., 1995. Initial colonization of the zebra mussel (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron: population recruitment, density and size structure. J. Great Lakes Res. 21, 417–434.

Nalepa, T.F., Schloesser, D.W., Pothoven, S.A., Hondorp, D.W., Fanslow, D.L., Tuchman, M.L., Fleischer, G.W., 2001. First finding of the amphipod *Echinogammarus ischnus* and the mussel *Dreissena bugensis* in Lake Michigan. J. Great Lakes Res. 27, 384–391.

Nalepa, T.F., Fanslow, D.L., Lang, G. a., 2009. Transformation of the offshore benthic community in Lake Michigan: Recent shift from the native amphipod *Diporeia* spp. to the invasive mussel *Dreissena rostriformis bugensis*. Freshw. Biol. 54, 466–479.

Nalepa, T.F., Fanslow, D.L., Pothoven, S.A., 2010. Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. J. Great Lakes Res. 36 (Supplement 3), 5–19.

Nalepa, T.F., V. Pavlova, W.H. Wong, J. Janssen, C.S. Houghton, and K. Mabrey. 2013. Variation in the quagga mussel (*Dreissena rostriformis bugensis*), with emphasis on the deepwater morphotype in Lake Michigan. In Quagga and Zebra Mussels: Biology, Impacts, and Control, Second Edition. T.F. Nalepa, and D.W. Schlosser (Eds.). CRC Press, Boca Raton, FL, pp. 315-329

Nichols, S.J., 1993. Spawning of zebra mussels (*Dreissena polymorpha*) and rearing of veligers under laboratory conditions. In: Nalepa, T.F., Schloesser, D.W. (Eds.), Zebra Mussels: Biology, Impacts, and Control. CRC Press, Boca Raton, FL, pp. 315–329.

Peyer, S.M., Hermanson, J.C., Lee, C.E., 2010. Developmental plasticity of shell morphology of quagga mussels from shallow and deep-water habitats of the Great Lakes. J. Exp. Biol. 213, 2602–9.

Peyer, S.M., Hermanson, J.C., Lee, C.E., 2011. Effects of shell morphology on mechanics of zebra and quagga mussel locomotion. J. Exp. Biol. 214, 2226–36.

Pothoven, S. A., Fahnenstiel, G.L., 2013. Recent change in summer chlorophyll a dynamics of southeastern Lake Michigan. J. Great Lakes Res. 39, 287–294.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Ram, J.L., Nichols, S.J., 1993. Chemical regulation of spawning in the zebra mussel (*Dreissena polymorpha*). In: Nalepa, T.F., Schlowsser, D.W. (Eds.), Zebra Mussels: Biology, Impacts, and Control. CRC Press, Boca Raton, FL, pp. 315–329.

Roe, S.L., MacIsaac, H.J., 1997. Deepwater population structure and reproductive state of quagga mussels (*Dreissena bugensis*) in Lake Erie. Can. J. Fish. Aquat. Sci. 54, 2428–2433.

Schwaebe, L., Acharya, K., Nicholl, M., 2013. Comparative efficacy of *Dreissena rostriformis bugensis* (Bivalvia: Dreissenidae) spawning techniques. Aquat. Invasions 8, 45–52.

Speziale, B.J., Schreiner, S.P., Giammatteo, P.A., Schindler, J.E., 1984. Comparison of N, N dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. Can. J. Fish. Aquat. Sci. 41, 1519–1522.

Sprung, Ml, 1984. Physiological energetics of mussel larvae (*Mytilus edulis*) 1. Shell growth and biomass. Mar. Ecol. Prog. Ser. 17, 283–293.

Stoeckmann, A., 2003. Physiological energetics of Lake Erie dreissenid mussels: a basis for the displacement of *Dreissena polymorpha* by *Dreissena bugensis*. Can. J. Fish. Aquat. Sci. 60, 126–134.

Strayer, D.L., Malcom, H.M., 2006. Long-term demography of a zebra mussel (*Dreissena polymorpha*) population. Freshw. Biol. 51, 117–130.

Vanderploeg, H.A., Nalepa, T.F., Jude, D.J., Mills, E.L., Holeck, K.T., Liebig, J.R., Grigorovich, I.A., Ojaveer, H., 2002. Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. Can. J. Aquat. Sci. 59, 1209–1228.

Vanderploeg, H., Liebig, J., 2010. *Dreissena* and the disappearance of the spring phytoplankton bloom in Lake Michigan. J. Gt. Lakes Res. 36, 50–59.

Vander Zanden, M.J., Hansen, G.J. a., Higgins, S.N., Kornis, M.S., 2010. A pound of prevention, plus a pound of cure: Early detection and eradication of invasive species in the Laurentian Great Lakes. J. Great Lakes Res. 36, 199–205

Wacker, A., Von Elert, E., 2008. Body size and food thresholds for zero growth in *Dreissena polymorpha*: a mechanism underlying intraspecific competition. Freshw. Biol. 53, 2356–2363.

Wilson, K., Howell, E., Jackson, D., 2006. Replacement of zebra mussels by quagga mussels in the Canadian nearshore of Lake Ontario: the importance of substrate, round goby abundance, and upwelling. J. Great Lakes Res. 32, 11–28.