Characteristics of a refuge for native freshwater mussels (Bivalvia: Unionidae) in Lake St. Clair

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A B S T R A C T

The Lake St. Clair delta (∼100 km²) provides an important refuge for native freshwater mussels (Unionidae) wherein 22 of the ∼35 historical species co-occur with invasive dreissenids. A total of 1875 live unionids representing 22 species were found during snorkeling surveys of 32 shallow (∼1 m) sites throughout the delta. Richness and density of unionids and zebra mussel infestation rates varied among sites from 3 to 13 unionid species, 0.02 to 0.12 unionids/m², and ∼1 to 35 zebra mussels/unionid, respectively. Zebra mussel infestation of unionids in the delta appears to be mitigated by dominant offshore currents, which limit densities of zebra mussel veligers in nearshore compared to offshore waters (13,600 vs. 28,000/m³, respectively). Glycogen concentrations in the tissues of a common and widespread species in the delta (Lampsilis silquoidae) suggest that zebra mussels may be adversely affecting physiological condition of unionids in a portion of the Lake St. Clair delta. Physiological condition and community structure of unionids within the delta may also be influenced by differences in food quantity and quality resulting from the uneven distribution of water flowing from the St. Clair River. The delta likely supports the largest living unionid community in the lower Great Lakes and includes several species that have been listed as Endangered or Threatened in Canada and/or the state of Michigan, making it an important refuge for the conservation of native unionids.

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Introduction

In the Great Lakes, declines in native unionids due to the impacts of zebra mussels (Dreissena polymorpha Pallas, 1771) have been well-documented (e.g., Schloesser and Nalepa 1994, Nalepa et al., 1996, Schloesser et al., 2006). However, several “refuge” sites for unionids have been found in nearshore wetland habitats of Lake Erie where zebra mussels are present, but at low densities. A viable community of 21 species was found in Metzger Marsh along the south shore of Lake Erie in 1996 (Nichols and Wilcox, 1997), and a community of 21 species was found in nearby Crane Creek Marsh in 2001 (Bowers and de Szalay 2004). Thompson Bay, the outer harbor of Presque Isle Bay, supports a smaller community of only nine species, and annual monitoring between 1992 and 2006 suggests that the community is stable (E.C. Masteller, Pennsylvania State University. pers. comm. 2008). The locations, characteristics, and ultimate significance of these refuges are of considerable interest for unionid conservation.

Zanatta et al. (2002) discovered an important refuge for unionids in the Lake St. Clair delta, where 22 of ∼35 historical species were found alive between 1999 and 2001. This refuge falls mainly within the territory of the Walpole Island First Nation. Eight of the species found are designated as Endangered or Threatened in Canada and/or Michigan and a ninth species, the Northern River Molluscan Unionid (Epioblasma torulosa rangiana Lea, 1838), is listed as Endangered in the US, Canada and Michigan (COSEWIC, 2007a; Michigan DNR, 2007; U.S. Fish and Wildlife Service 2007). Densities of unionids in the Lake St. Clair delta are low, ranging from 0.03 to 0.07 unionids/m² (Zanatta et al. 2002), compared to an average density of 2 unionids/m² reported for offshore waters of Lake St. Clair before the zebra mussel invasion in 1986 (Nalepa and Gauvin 1988). However, the Lake St. Clair refuge is much larger than any of the other refuge sites discovered to date in...
Lake Erie (∼ 100 km² vs. 1 to 3 km²) and, as such, it sustains some of the largest remaining populations of unionids in the lower Great Lakes. For example, the estimated size of the Villusia iris (Lea, 1829) population in the delta is ∼ 190,000 individuals (COSEWIC, 2006).

Infestation rates of zebra mussels on unionids in the Lake St. Clair delta are variable and generally higher than those found in other refugia in Lake Erie. Average rates (# zebra mussels/unionid) were ∼ 1 in Metzger Marsh (Nichols and Ambergh 1999), zero in Thompson Bay (Schloesser and Masteller 1999) and 4 in Crane Creek Marsh (Bowers and de Szalay 2004), whereas average infestation rates in the delta ranged from 3 to 47 (Zanatta et al. 2002). In Metzger Marsh, the limited infestation rates are likely due to the presence of soft sediments, which allow unionids to burrow, thereby smothering any zebra mussel veligers. The low infestation rates in Thompson Bay and Crane Creek Marsh are attributed to water-level fluctuations that expose zebra mussels and cause them to either release voluntarily from unionids or perish (Schloesser and Masteller 1999, Bowers and de Szalay 2005). Zanatta et al. (2002) speculated that low infestation rates of unionids in the nearshore waters of Lake St. Clair delta, relative to those recorded in the open waters of the lake (Gillis and Mackie 1994), may be the result of fluctuations in the number of zebra mussel veligers reaching and settling in the shallow areas of the delta due to variations in wind direction, currents and water levels.

Although the zebra mussel infestation rates reported in the delta by Zanatta et al. (2002) are well below the lethal threshold of 100 zebra mussels/unionid reported by Ricciardi et al. (1995) and Schloesser et al. (1996), a subsequent study determined that significant declines in unionid density can occur when the mean infestation rate exceeds 10 zebra mussels/unionid (Ricciardi et al., 1996). Unionid mortality in some systems has also been attributed to increased competition for food resources by zebra mussels (Strayer and Smith, 1996). Assessing the effects of low levels of zebra mussel infestation on the physiological condition of unionids may help to determine if these remnant Great Lakes populations are stable.

Effects of stressors, like zebra mussels, may adversely affect the relative condition of unionids at levels that are lower than those that cause mortality. Thus, researchers often look to sublethal measures, such as those influencing physiology, biochemistry and reproduction, because these measures may be predictive of future mortality. Glycogen is the principal storage form of carbohydrates in many aquatic invertebrates (Stetten and Stetten 1960, De Zwaan and Zandee 1972, Hummel et al. 1989) and has been used as an indicator of physiological condition in unionids. For example, glycogen has been used as an indicator of stress resulting from emersion (Greseth et al. 2003), parasitic infestations (Jokela et al. 1993), relocation and quarantine (Patterson et al. 1997, Naimo et al. 1998), and zebra mussel infestation (Haag et al. 1993, Hallac and Marsden 2000).

Changes in total lipid and/or individual fatty acid concentrations in organisms can reveal broad scale changes in ecosystem function (Hedrick et al. 2006, 2008). Fatty acids have been successfully used as trophic markers (reviewed in Dalsgaard et al., 2003). Because many fatty acids found in the diet are conserved within the tissues of consumers they have seen widespread use as biomarkers of feeding both in the short (e.g. Brett et al. 2006; Iverson in press) and long term (e.g. Hedrick et al. 2008). Traditional methods for determining diet (e.g., gut content analyses, behavioral studies) are time consuming, labor intensive and generally do not integrate the effects of feeding temporally and/or spatially. Fatty acids are helpful in this respect because they integrate the effects of feeding over time. They have also been extensively used as indices of condition in a wide range of aquatic organisms (Arts and Wainman 1999, Schlechtriem et al. 2008) and, occasionally, in marine mussels (Hellou and Law 2003, Alkanani et al. 2007). Although total lipid content has been used to assess the condition of unionids (Haag et al. 1993), using specific fatty acids in such contexts has not been done before.

The Walpole Island First Nation and Environment Canada’s Water Science and Technology Directorate formed a 3-year partnership in 2003 to develop and implement an action plan to conserve and recover unionid communities of the Lake St. Clair delta. The objectives were to: a) document the composition of unionid communities throughout the shallow (< 1.5 m) waters of the Lake St. Clair delta, b) determine the spatial distribution and abundance of zebra mussels veligers, c) evaluate the effects of water currents on the distribution of zebra mussel veligers and, d) examine variation in the physiological condition of unionids across the delta as they relate to the rate of zebra mussel infestation.

Methods

Study area

The Lake St. Clair delta is located at the mouth of the St. Clair River where it enters the northeast end of Lake St. Clair (Fig. 1). The delta is bisected by the Canada/US border between the Province of Ontario and the State of Michigan. The Canadian portion of the delta falls mainly within the territory of the Walpole Island First Nation (WIFN), which contains > 12,000 ha of wetlands and is one of the largest wetland complexes in the Great Lakes Basin (The Nature Conservancy 1995). More than 50 plant and animal species listed as Canadian Species at Risk occur on the lands and in the waters of the WIFN (Bowles 2005). In contrast, the US portion of the delta is highly urbanized with hardened shorelines (Jaworski and Raphael 1976). Average annual discharge of the St. Clair River is ∼ 5000 m³/s (Lake St. Clair Canadian Watershed Coordination Council 2005). The flow of water from the river is divided into three main channels in the upper part of the delta and a number of secondary channels in the lower delta. The eastern (Canadian) section of Lake St. Clair receives only 8% of the inflow from the St. Clair River (Environment Canada, et al. 1984). A dredged navigation channel crosses the lake along the international border, and water masses on either side of the channel rarely mix (Lake St. Clair Canadian Watershed Coordination Council 2005). The southern (Canadian) water mass is warmer and more productive, whereas the northwestern (US) water mass, which receives most of its water from Lake Huron, is cooler and less productive (Leach 1980).

Assessment of unionid communities in the delta

Thirty two sites were surveyed for unionids, 18 quantitatively and 14 semi-quantitatively (Fig. 1). Surveys were conducted at depths of 0.5 to 1.5 m where previous surveys showed most live unionids would be found (Zanatta et al. 2002). Individual sites were selected for survey using several criteria. First, quantitative sampling was repeated at nine sites that had been previously surveyed by Zanatta et al. (2002). The other 9 quantitative sites and the 14 semi-quantitative sites were selected to distribute sampling effort across the shallow bays of the delta and in a few instances were based on aboriginal knowledge of areas known to support unionids. Only locations where live unionids were observed were selected for intensive quantitative surveys. Given that sites were not chosen randomly, these data should not be viewed as representative of the entire delta. Quantitative surveys were conducted from July 21 to August 8, 2003 at nine sites in Canadian waters (Sites 1–7, 15 and 18) and nine sites in US waters (Sites 8–14 and 16–17) using the following “circle-plot” sampling technique devised by Zanatta et al. (2002). At each site, three transects spaced ∼ 25 m apart perpendicular to shore were searched by a two-person team consisting of a snorkeler and a helper. The snorkeler swims until a live unionid was encountered, at which point the helper moved around the stake in circles then swam around the stake in circles.
of decreasing diameter until the entire 65 m² area had been searched and all visible live unionids had been collected and placed in a mesh bag for examination. This procedure was repeated until each team had surveyed 10 plots along each transect. At sites where unionid densities were very low, some or all snorkelers were unable to survey their full 10 plots before either reaching shore or entering waters that were too deep to search effectively, resulting in smaller search areas at these sites (Table 2). Locations of transects were marked using a hand-held GPS unit. The unionids collected were identified to species, counted and then maximum shell lengths were measured to the nearest mm using vernier calipers. Zebra mussels were removed from three randomly selected individuals of each unionid species from each site and counted to estimate the infestation rate. Shell lengths of zebra mussels were also measured.

The “circle-plot” technique would likely overestimate the actual density of unionids at a site if the unionids were exhibiting an aggregated distribution in the rather uniform “sand flat” habitat of the delta. On the other hand, the technique may also underestimate densities at a site because it does not detect burrowed adults and juveniles. As unionid densities are very low in the delta, this method generated data with a reasonable amount of effort and established a baseline from which future surveys using the same method could be directly compared.

Semi-quantitative surveys were conducted from July 21 to August 8, 2003 and August 9 and 10, 2005 at six sites in Canadian waters and eight sites in US waters using a timed search technique. Surveys involved one to four snorkelers who spread out and randomly searched the area for unionids for times ranging from 0.25 to 4 person-hours. Live unionids were identified to species, measured to the nearest mm, and counted. Data from all 32 quantitative and semi-quantitative sites were combined only to establish the number and composition of species in the delta. All other analyses were performed using the data obtained from the 18 quantitative survey sites. All unionids collected during these surveys were returned to the lake alive except for those sacrificed for physiological condition analyses.

The role of water movement in transport of zebra mussel veligers

The rates of zebra mussel infestation on unionids depend, in part, on the number of zebra mussel veligers to which the unionids are exposed (Martel et al. 1994). Unionids continue to survive in the delta long after they were extirpated from the offshore waters of the lake sometime between 1994 and 1997 (Nalepa et al. 2001), suggesting that the number of veligers reaching and/or settling in the delta are low. To test this hypothesis, four transects were established in different areas of the delta (Fig. 2). Veligers were collected from stations with water depths of 4 m (offshore), 2 m (intermediate), and <1 m (nearshore) along each transect on seven occasions between

Fig. 1. Locations of 32 sites surveyed for unionids in the Lake St. Clair delta in 2003 and 2005. Sites surveyed quantitatively (n = 18) are indicated by circles containing the site number; sites surveyed semi-quantitatively (n = 14) are indicated by black triangles.
June 3 and August 27, 2003. In 2004, veligers were collected on six occasions between June 3 and August 11 at the same stations along transects 2 and 3 and at four additional inshore stations near Sites 1 and 18 where the highest densities of unionids had been found in 2003 (see Fig. 2). Samples were collected in triplicate from each of the stations using a 12 L capacity Schindler-Patalas trap with 40 μm mesh and preserved in ∼70% ethanol. In the lab, a 50–90 ml aliquot of each sample was poured into a square-gridded Petri dish. Six to 12 of the 36 1-cm² squares were randomly selected, and all veligers within each square were enumerated and sorted into size classes representing the five stages of the veliger life cycle, including the three planktonic forms: pre-D form (<80 μm), D form (81–150 μm), and veliconcha (151–250 μm) and the two settling forms: pediveliger (251–300 μm) and plantigrade (N300 μm). Veliger counts were averaged by station for each year and log-transformed for statistical analyses.

An acoustic Doppler current profiler (ADCP) and three optical backscatter sensors (OBS) with data loggers were installed on the nearshore side of a sandbar off Walpole Island (Fig. 2) to investigate the role of water movement in transporting zebra mussel veligers into the delta. The ADCP measured current velocity and direction while the OBS measured turbidity to indicate sediment re-suspension events. The equipment was deployed by SCUBA divers at 2.0 m depth on August 22, 2003, and remained operational until it was removed on November 17, 2003. ADCP data were plotted to illustrate current magnitude and direction during the 62 day monitoring period. Meteorological data (water temperature, barometric pressure, wave height and period, and wind speed) were obtained from Environment Canada ODAS weather buoy # 45147 in Lake St. Clair to monitor storm events over the same 62 day period. The weather buoy is located in the centre of the lake approximately 11 km from the location of the ADCP.

Physiological condition analyses

Foot and mantle tissues were collected from 54 male Lampsilis siliquoidea (Barnes, 1823) at 14 of the 18 quantitatively-surveyed sites in 2003. Tissues were collected from four specimens at each site except for Sites 12 and 15 where only three animals were found. L. siliquoidea was not present at Sites 2, 5, 6, and 7. Tissues were immediately frozen on dry ice and stored at −80 °C until they could be analyzed. L. siliquoidea was selected because it is widely distributed in the delta, and males were chosen to minimize variation due to reproductive condition. Foot and mantle tissues were combined for all physiological condition analyses. These data were used to assess relationships between physiological condition indicators (glycogen or fatty acids) and the rate of zebra mussel infestation on unionids, and...
to determine if there were differences in the relative condition of mussels across the delta.

Glycogen

For most samples, a 10.0 ± 2.0 mg aliquot of tissue was analyzed for glycogen using the alkaline digestion and phenol-sulfuric acid spectrophotometric method (Naimo et al. 1998, Naimo and Monroe 1999, Monroe and Newton 2001). About 3% of samples were smaller, so a 5.0 ± 2.0 mg aliquot was used in order to leave sufficient material for quality assurance and quality control. The accuracy of glycogen determinations was quantified by the use of procedural blanks, replicates of an in-house reference material (Naimo et al. 1998), triplicate analyses of four aqueous calibration standards, triplicate analyses of randomly selected tissue samples, and triplicate analyses of known additions. As a measure of precision, the relative standard deviation of triplicate analyses of either known additions or tissue samples was estimated according to APHA et al. (1995). The relative standard deviation of 10 triplicate known additions averaged (±SD) 19 ± 9%. Bias associated with glycogen determinations was estimated by recovery of known additions (APHA et al., 1995). Mean recovery in 10 composite samples was 67 ± 16%. Our recovery estimates are similar to those of Naimo and Monroe (1999), who reported 70–75% recovery of known addition samples. The lower recoveries may be related to the use of known additions, where background concentrations in non-spiked samples are considerably more variable, than in matrix standards where variation is reduced due to the use of a standardized tissue sample.

Fatty acids

Analysis included three procedures: gravimetric extraction, derivitization, and quantification on a gas chromatograph (GC) following the methods described in Zellmer et al. (2004). Fatty acid methyl esters (FAME) were identified using Supelco’s 37 component FAME standard (#47885-U) by comparing peak retention times between samples and standards. The FAME standard was run as a 4-point standard curve with each set of twenty samples. This standard curve was used also to quantify the amount of each fatty acid in a sample. Also, an internal standard (5α-Cholestane; Sigma-Aldrich; #C8003) was added to the tissue before extraction to estimate percent recovery. Additional single fatty acid standards were used to expand the range of quantifiable FAME to include other important fatty acid (i.e., docosapentaenoic acid; 22:5n-3) not included in the 37 component FAME standard. Fatty acid results were reported as μg FAME/mg dry mass of tissue.

Statistical analyses

For each of the three most common species, differences in length-frequency distributions between individuals collected in Canadian and US waters were tested using a two-sample Kolmogorov–Smirnov test. Differences in the number of zebra mussels attached to unionids at quantitatively surveyed sites were assessed using one-way ANOVA. Differences in the densities of zebra mussel veligers both among and within transects were assessed using two-way ANOVA with post-hoc Tukey pairwise comparisons. Relationships between unionid density and average zebra mussel infestation at a site and between glycogen or fatty acid concentrations in L. siliquoidea and the number of zebra mussels attached to their shells were assessed using Pearson correlations. Differences in the slope of the relationship between zebra mussel infestation rate and glycogen concentration in L. siliquoidea between Canadian and US waters were assessed using 2-way ANOVA. The distribution of fatty acid markers in L. siliquoidea across the delta was investigated using discriminant analysis to identify the best subset of fatty acid markers needed to separate unionids among the major bays of the delta. All analyses were conducted using SYSTAT® v. 11 and, where necessary, data were transformed using the natural logarithm to meet the assumptions of normality.

Results

Assessment of unionid communities in the delta

Unionid surveys

A total of 1875 unionids of 22 species were found at 32 sites in the St. Clair delta in 2003 and 2005 (Table 1). Eight of these species are listed as Endangered or Threatened in one or more jurisdictions. Members of the sub-families Lampsiilinae and Ambleminae comprised 76% and 22%, respectively, of all unionids collected, whereas members of the sub-family Anodontinae accounted for only 2%. Lampsiilis siliquoidea was the most abundant and frequently encountered

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Subfamily</th>
<th>Relative abundance (%)</th>
<th>Percent occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lampsiilis siliquoidea (Barnes, 1823)</td>
<td>Lampsiilinae</td>
<td>47.49</td>
<td>90.6</td>
</tr>
<tr>
<td>Fusconaia flava (Rafinesque, 1820)</td>
<td>Ambleminae</td>
<td>12.82</td>
<td>68.8</td>
</tr>
<tr>
<td>Lampsiilis cardium (Rafinesque, 1820)</td>
<td>Lampsiilinae</td>
<td>12.07</td>
<td>78.1</td>
</tr>
<tr>
<td>Pleurobema sintoxia (Rafinesque, 1820)</td>
<td>Ambleminae</td>
<td>6.84</td>
<td>34.4</td>
</tr>
<tr>
<td>Villosa iris (Lea, 1829)</td>
<td>Lampsiilinae</td>
<td>4.86</td>
<td>50.0</td>
</tr>
<tr>
<td>Petalilus olatus (Say, 1817)</td>
<td>Lampsiilinae</td>
<td>4.81</td>
<td>59.4</td>
</tr>
<tr>
<td>Ligumia nasuta (Say, 1817)</td>
<td>Lampsiilinae</td>
<td>4.43</td>
<td>25.0</td>
</tr>
<tr>
<td>Ambrema plicata (Say, 1817)</td>
<td>Ambleminae</td>
<td>1.98</td>
<td>31.3</td>
</tr>
<tr>
<td>Leptidea fragilis (Rafinesque, 1820)</td>
<td>Lampsiilinae</td>
<td>0.75</td>
<td>21.9</td>
</tr>
<tr>
<td>Pygymoide granulid (Say, 1828)</td>
<td>Ambeiniae</td>
<td>0.64</td>
<td>15.6</td>
</tr>
<tr>
<td>Elliptio dilatata (Rafinesque, 1820)</td>
<td>Ambleminae</td>
<td>0.59</td>
<td>21.9</td>
</tr>
<tr>
<td>Obovaria subrotunda (Rafinesque, 1820)</td>
<td>Ambleminae</td>
<td>0.59</td>
<td>18.8</td>
</tr>
<tr>
<td>Lampsiilis fasciolata (Rafinesque, 1820)</td>
<td>Lampsiilinae</td>
<td>0.53</td>
<td>18.8</td>
</tr>
<tr>
<td>Lamigonia costata (Rafinesque, 1820)</td>
<td>Anodontinae</td>
<td>0.43</td>
<td>12.5</td>
</tr>
<tr>
<td>Anodontoides fassicanus (Lea, 1834)</td>
<td>Anodontinae</td>
<td>0.27</td>
<td>6.3</td>
</tr>
<tr>
<td>Ligumia recta (Lamarck, 1819)</td>
<td>Lampsiilinae</td>
<td>0.27</td>
<td>6.3</td>
</tr>
<tr>
<td>Strophites undulatus (Say, 1817)</td>
<td>Anodontinae</td>
<td>0.21</td>
<td>12.5</td>
</tr>
<tr>
<td>Aksomida margina (Say, 1818)</td>
<td>Anodontinae</td>
<td>0.16</td>
<td>9.4</td>
</tr>
<tr>
<td>Psychobranchus fasciolaris (Rafinesque, 1820)</td>
<td>Lampsiilinae</td>
<td>0.11</td>
<td>6.3</td>
</tr>
<tr>
<td>Quadruma pusilia (Lea, 1831)</td>
<td>Ambleminae</td>
<td>0.05</td>
<td>3.1</td>
</tr>
<tr>
<td>Quadruma quadru (Rafinesque, 1820)</td>
<td>Ambeiniae</td>
<td>0.05</td>
<td>3.1</td>
</tr>
<tr>
<td>Truncilla donaciformis (Lea, 1829)</td>
<td>Lampsiilinae</td>
<td>0.05</td>
<td>3.1</td>
</tr>
</tbody>
</table>

a Endangered in Canada.
b Endangered in Michigan.
c Threatened in Canada (Great Lakes-Western St. Lawrence population).
d Threatened in Michigan.
The length distributions were significantly different in Canadian and US waters (12, 13).

Fusconaia species, followed by Fusconaia flava (Rafinesque, 1820), Lampsilis cardium (Rafinesque, 1820) and Pleurobema sintoxia (Rafinesque, 1820). Species richness at the 18 quantitative sites ranged from 3 to 13 species/site, and mean density ranged from 0.02 to 0.12 unionids/m² (Table 2).

Unionid community structure varied throughout the study area. There were differences in species composition among sites in Canadian and US waters. Sites in Canadian waters supported more species than sites in US waters (19 vs. 13), although average species richness did not differ (t-test; \( p = 0.154 \)) between Canadian and US waters (8 vs. 6). Canadian waters also supported a 16% higher overall density of unionids (0.050/m² vs. 0.043/m²) and more Endangered and Threatened species (100 specimens of 5 species vs. 77 specimens among 6 species) in total veliger densities among the offshore, intermediate and nearshore stations in the delta in 2003 (90%) and 2004 (87%), while only 7% of the veligers in 2003 and 10% in 2004 were settling stage pediveligers. Veliger densities at offshore stations in 2003 (28,000 ± 5042 larvae/m³) were significantly higher than those at the intermediate (13,408 ± 3152/m³) and nearshore stations (13,581 ± 2720/m³), which did not differ from each other (Fig. 3).

Fig. 3. Mean densities (#/m³ ± SE) of zebra mussel veligers in water samples collected from offshore, intermediate, nearshore and inshore stations in the Lake St. Clair delta during the summers of 2003 (n = 28 at each location) and 2004 (n = 10 at offshore, intermediate, and nearshore stations and n = 15 at the inshore stations). Locations not connected by the same letter had significantly different veliger densities. Differences between years were not assessed.

Zebra mussel infestation

A total of 4605 zebra mussels were removed from the shells of ~20% of unionids collected from the quantitative sites. Overall, the mean infestation rate (±SE) across all sites was 15 ± 1 zebra mussels/uniorid. Infestation rates differed significantly among sites (\( F_{12,28} = 14.8; p < 0.001 \)), ranging from <1 at Site 1 to 35 at Site 6 (Table 2) but did not differ between Canadian and US waters (\( F_{11,42} = 0.09; p = 0.993 \)). There was a negative, marginally significant, correlation between mean density of unionids and mean rate of zebra mussel infestation (\( r = 0.18; p = 0.094 \)). The lengths of individual zebra mussels removed from unionids ranged from 2 to 38 mm. The size class distribution of attached zebra mussels, categorized into three length ranges (1–12, 13–24, and 25–40 mm), varied considerably across the delta (Table 2).

The role of water movement in transport of zebra mussel veligers

Zebra mussel veligers were present in the water column over the entire study period in both 2003 and 2004. Significant differences were observed in total veliger densities among the offshore, intermediate and nearshore stations in the delta in 2003 (\( F_{2,73} = 10.0; p < 0.001 \)) and 2004 (\( F_{3,42} = 7.9; p = 0.001 \)) and also among transects in 2003 and 2004 (\( F_{3,73} = 4.5; p = 0.004 \) and \( F_{1,42} = 9.7; p = 0.001 \) respectively). Veliger densities at offshore stations in 2003 (28,000 ± 5042 larvae/m³) were significantly higher than those at the intermediate (13,408 ± 3152/m³) and nearshore stations (13,581 ± 2720/m³), which did not differ from each other (Fig. 3).

Table 2

<table>
<thead>
<tr>
<th>Site</th>
<th>Area searched (m²)</th>
<th>Number of species</th>
<th>Mean density (#/m² ± SE)</th>
<th>Infestation rate (# uniorid ± SE)</th>
<th>% of zebra mussels in each length class (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1–12</td>
</tr>
<tr>
<td>US waters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1755</td>
<td>5</td>
<td>0.032 ± 0.003</td>
<td>11 ± 3</td>
<td>19</td>
</tr>
<tr>
<td>13</td>
<td>1950</td>
<td>6</td>
<td>0.042 ± 0.004</td>
<td>13 ± 3</td>
<td>9</td>
</tr>
<tr>
<td>17</td>
<td>1950</td>
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<td>0.048 ± 0.003</td>
<td>2 ± 0.5</td>
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<tr>
<td>16</td>
<td>1950</td>
<td>4</td>
<td>0.043 ± 0.007</td>
<td>9 ± 3</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>1950</td>
<td>6</td>
<td>0.027 ± 0.004</td>
<td>15 ± 2</td>
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<tr>
<td>14</td>
<td>1950</td>
<td>8</td>
<td>0.081 ± 0.010</td>
<td>27 ± 5</td>
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<tr>
<td>10</td>
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<tr>
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<td>0.039 ± 0.010</td>
<td>7 ± 2</td>
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<tr>
<td>8</td>
<td>1235</td>
<td>7</td>
<td>0.045 ± 0.010</td>
<td>29 ± 4</td>
<td>23</td>
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<td>Canadian waters</td>
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<td>0.7 ± 0.2</td>
<td>3</td>
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<tr>
<td>2</td>
<td>260</td>
<td>3</td>
<td>0.021 ± 0.005</td>
<td>8 ± 4</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>1950</td>
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<td>0.075 ± 0.008</td>
<td>15 ± 2</td>
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<tr>
<td>4</td>
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<td>8</td>
<td>0.033 ± 0.008</td>
<td>7 ± 2</td>
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<tr>
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<td>0.023 ± 0.003</td>
<td>27 ± 6</td>
<td>53</td>
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<tr>
<td>6</td>
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<td>0.021 ± 0.005</td>
<td>35 ± 15</td>
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</tr>
<tr>
<td>7</td>
<td>910</td>
<td>6</td>
<td>0.023 ± 0.004</td>
<td>21 ± 4</td>
<td>17</td>
</tr>
</tbody>
</table>

Sites arranged from west to east.

1 Zebra mussels not measured.
Eicosapentaenoic acid (EPA) 20:5n-3 18.3
Arachidonic acid (ARA) 20:4n-6
Oleic acid 18:1n-9
Heptadecanoic acid 17:0
α-Palmitoleic acid 16:1n-7 8.1 4.7
Pentadecanoic acid 15:0 2.8 8.6

Fig. 4. Relationships between glycogen concentrations (mg/g dry weight) in combined foot and mantle tissues of individual male Lampsilis siliquoidea collected from the Lake St. Clair delta in 2003 and the number of zebra mussels attached to their shells. Lines represent regressions for specimens collected from Canadian sites (solid line) and US sites (dashed line).

Glycogen concentrations in L. siliquoidea ranged from 18 to 167 mg/g across the delta (Fig. 4). Highest mean glycogen concentrations observed were in specimens collected from Site 1 (125 ± 14.3 mg/g) and Site 18 (82.5 ± 13.0 mg/g)—sites which also supported the most diverse and abundant unionid communities. Across the delta, there was a negative, marginally significant, correlation between mean glycogen concentration and zebra mussel infestation rate \( r = -0.258; \ p = 0.084 \). However, there was a significant difference in the mean concentration of glycogen in L. siliquoidea at Canadian sites (75.9 ± 6.1 mg/g; \ n = 19 \) compared to US sites (40.0 ± 10.1 mg/g; \ n = 35 \) \( t \)-test; \( p < 0.001 \) and differences were also apparent in the slope of the relationship between glycogen concentration and zebra mussel infestation rate in Canadian vs. US sites \( F_{1,42} = 10.4; \ p = 0.002 \) (Fig. 4). At Canadian sites, there was a negative correlation between zebra mussel infestation rate and glycogen concentration \( r = -0.667; \ p = 0.007 \), whereas at US sites the relationship was not significant \( r = 0.079; \ p = 0.672 \).

Table 3
Canonical discriminant coefficients of the 9 fatty acids retained in the discriminant analysis of the fatty acid composition of Lampsilis siliquoidea in the Lake St. Clair delta in 2003

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Molecular formula</th>
<th>Factor 1</th>
<th>Factor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentadecanoic acid</td>
<td>15:0</td>
<td>2.8</td>
<td>8.6</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>16:1n-7</td>
<td>8.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Heptadecanoic acid</td>
<td>17:0</td>
<td>-3.0</td>
<td>9.8</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18:1n-9</td>
<td>-13.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18:2n-6</td>
<td>-2.1</td>
<td>32.5</td>
</tr>
<tr>
<td>α-linolenic acid (ALA)</td>
<td>18:3n-3</td>
<td>-12.8</td>
<td>24.7</td>
</tr>
<tr>
<td>Arachidonic acid (ARA)</td>
<td>20:4n-6</td>
<td>-15.8</td>
<td>-10.1</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA)</td>
<td>20:5n-3</td>
<td>18.3</td>
<td>-12.6</td>
</tr>
<tr>
<td>Docosahexaenoic acid (DHA)</td>
<td>22:5n-3</td>
<td>20.5</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Fig. 5. Canonical scores plot derived from a discriminant analysis of the concentrations of 9 fatty acids in the foot and mantle tissues of male Lampsilis siliquoidea grouped by the bay in which they were collected from the Lake St. Clair delta in 2003. Filled symbols are from animals collected in Canadian waters of the delta and hollow symbols from animals collected from US waters. The centroid for each 75% confidence ellipse is denoted by an X.

Physiological condition analyses

Glycogen
Glycogen concentrations in L. siliquoidea ranged from 3.8 to 9.0%. Total lipid content of unionids was not correlated with zebra mussel infestation \( r = -0.020; \ p = 0.856 \) at sites across the delta. There was no significant correlation between zebra mussel infestation and any individual fatty acid. However, because of the established role of diet as a key factor influencing fatty acid profiles, the spatial distribution of fatty acid profiles in unionids across the delta was investigated. Individual L. siliquoidea were grouped based on their occurrence in one of the five major bays of the delta (Fig. 1).

Table 3
Canonical discriminant coefficients of the 9 fatty acids retained in the discriminant analysis of the fatty acid composition of Lampsilis siliquoidea in the Lake St. Clair delta in 2003

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</tr>
<tr>
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<td>17:0</td>
<td>-3.0</td>
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<tr>
<td>Linoleic acid</td>
<td>18:2n-6</td>
<td>-2.1</td>
<td>32.5</td>
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<tr>
<td>α-linolenic acid (ALA)</td>
<td>18:3n-3</td>
<td>-12.8</td>
<td>24.7</td>
</tr>
<tr>
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<td>20:4n-6</td>
<td>-15.8</td>
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<td>22:5n-3</td>
<td>20.5</td>
<td>2.1</td>
</tr>
</tbody>
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Fatty acids
Mean total lipid levels (% dry weight) in L. siliquoidea ranged from 3.8 to 9.0%. Total lipid content of unionids was not correlated with zebra mussel infestation \( r = -0.020; \ p = 0.856 \) at sites across the delta. There was no significant correlation between zebra mussel infestation and any individual fatty acid. However, because of the established role of diet as a key factor influencing fatty acid profiles, the spatial distribution of fatty acid profiles in unionids across the delta was investigated. Individual L. siliquoidea were grouped based on their occurrence in one of the five major bays of the delta (Fig. 1). Nine fatty acids were retained in the discriminant analysis (Table 3). The first two factors accounted for 94% of the dispersion and clearly separated unionids from the two Canadian bays (Horseshoe and Chematogan) from each other and from the three US bays (Anchor, Goose, and Muscamoot), which grouped together (Fig. 5).

Discussion
Live unionids were found at all but one of the 32 sites surveyed in the shallow waters (<1.5 m) of the Lake St. Clair delta. Unionid densities averaged 0.05 ± 0.007/m² at the 18 quantitatively surveyed sites, which is the same as the average density of 0.05/m² reported for the delta in 2001 (Zanatta et al. 2002). Unionid densities have been reported for only one other Lake Erie refuge. Bowers and de Szalay (2004) reported a mean density of 0.01/m² in Crane Creek Marsh; however, in their study densities were determined using sampling plots dispersed randomly throughout an area deemed to contain suitable habitat for unionids. Given the difference in the sampling method used in their study, a direct comparison with the densities in the Lake St. Clair delta is not possible.

The three most abundant species in 2003 and 2005 (L. siliquoidea, F. fluvo and L. cardium) were also the most abundant in 1999–2001 (Zanatta et al. 2002). In the present study, the proportion of the unionid community in each of the subfamilies Lampsilinae, Amblyminae and Anodontinae was 76%, 22% and 2%, respectively, which is similar to the 80% Lampsilinae, 14% Amblyminae, and 6% Anodontinae reported by Nalepa and Gauvin (1988) for offshore waters of Lake St. Clair prior to the establishment of zebra mussels. As Lampsilinae and Anodontinae mussels are thought to be more affected by zebra mussel infestation than Amblymines (Schloesser et al. 1998), the dominance of Lampsilines in the unionid community of the shallow waters of the
The rates of zebra mussel infestation of unionids in refuge in Lake Erie are near zero either because unionids are able to shed their load of attached zebra mussels actively by burrowing in soft sediments (Nichols and Wilcox 1997; Schloesser et al. 1997) or the zebra mussels are killed or released from unionids when water-level fluctuations cause them to be exposed (Schloesser and Masteller 1999; Bowers and de Szalay 2005). The average number of zebra mussels attached to unionids declined from 61 in 1999 to 31 in 2000 to 17 in 2001 (Zanatta et al. 2002), and to 15 in this study, a trend which is consistent with observed declines in the density of zebra mussels at other sites in Lake St. Clair between 1994 and 1997 (Naleza et al. 2001). Although declining, infestation rates in the delta are still higher than those observed in the Lake Erie refuge. Only the unionids at Site 1 showed evidence of periodic shedding of attached zebra mussels, i.e., unionids at this site had almost no zebra mussels attached to them but most had the remnants of byssal threads on their shells. It was noted that substrates at Site 1 were less consolidated than at other sites and were easily disturbed during snorkeling; thus, it seems likely that unionids at this location may be able to burrow and rid themselves of attached zebra mussels. At most other sites in the delta, the majority of zebra mussels attached to unionoids were < 24 mm in length and thus were either in their first or second year of life based on the growth estimates for Lake St. Clair (Mackie 1991). Zebra mussels with lengths > 24 mm, which would likely be > 2 years old (Mackie 1991), were found attached to unionids at all but three of the sites surveyed quantitatively. The presence of zebra mussels from several age classes attached to unionoids, suggests that unionoids were not periodically shedding zebra mussels over much of the delta area and that some other mechanism(s) are likely responsible for the low rates of infestation in the delta.

The direction and rate of water flow in the delta may be limiting zebra mussel infestation of unionoids. Densities of zebra mussel veligers were consistently lower at sites nearest the shore in 2003 and 2004. Lower densities of veligers in the water column have been shown to result in lower settlement rates of zebra mussels onto artificial substrates (Martel et al. 1994); thus, zebra mussel veliger settlement rates in the delta are likely lower in waters ~ 1.5 m deep where live unionoids occur. The direction of water flow and predominant wind direction are both from the north-west, tending to move water and particulate matter, including planktonic veligers, out of the delta. This flow pattern reverses when the wind direction changes and blows from the south or southeast and persists long enough to push water into the delta, coinciding with re-suspension of sediments (as measured by increased turbidity). During such events in Lake Erie, Martel (1993) demonstrated increased drift and settlement of postmetamorphic zebra mussels, which were also re-suspended into the water column. Postmetamorphic zebra mussels are thought to have a better chance of survival as they have already made the transformations necessary for the transition to benthic life (Martel 1993). Flow reversal events are one mechanism by which zebra mussel veligers and/or postmetamorphic zebra mussels could enter the shallow areas of the delta where the unionoids are found, but the low frequency of these flow reversals likely limits the exposure of unionoids to zebra mussels.

There were differences in the unionid communities found in Canadian and US waters of the delta. The unionoid community in US waters was less diverse and was dominated (~ 90% of abundance) by three species (L. siliquoides, V. iris and F. flava), whereas the unionoid community in Canadian waters was more diverse, with five species (L. siliquoides, F. flava, L. cardium, Ligumia nasuta (Say, 1817) and P. sintonix) making up 90% of the community by abundance. Unionoid densities, species richness, and the number of Endangered and Threatened species were also greater in Canadian waters of the delta. It is speculated that these differences are due, at least in part, to differences in productivity and land use on either side of the delta. The shipping channel, which bisects Lake St. Clair along the border, effectively splits the lake into less productive (US) and more productive (Canada) water masses (Leach 1980).

Although zebra mussel infestation rates in the delta are low, there is evidence that unionoids may be experiencing some negative effects. There was a weak negative correlation between unionoid density and zebra mussel infestation rate. There was also a significant negative correlation between glycogen concentration in L. siliquoides and the number of attached zebra mussels in Canadian waters of the delta. A similar pattern has been seen in other systems. For example, specimens of Ambplema pilica (Say, 1817) and Quadrula pustulosa (Lea, 1831) obtained from a heavily infested (> 350 zebra mussels/m²) reach of the Ohio River had significantly lower glycogen levels than specimens obtained from a lightly infested (< 5 zebra mussels/m²) reach (Patterson et al. 1997). Unlike in Canadian waters, the slope of the relationship between glycogen and zebra mussel infestation rate in US waters was not significantly different from zero, indicating that L. siliquoides in US waters had similar, low glycogen concentrations regardless of the rate of zebra mussel infestation. Although increased stress has been clearly linked with reductions in energy stores as glycogen in unionoids, the reductions in glycogen have not resulted in increased mortality (Haag et al. 1993, Newton et al. 2001). The inconsistency between glycogen concentration and the rate of zebra mussel infestation in Canadian and US waters may result from the ability of unionoids to slowly utilize stored glycogen during periods of food limitation and to maintain glycogen levels above 25% (on a dry wt basis) for ~ 200 days without food (Ellis and Calvin 1936). However, due to the lack of definitive physiological thresholds, it cannot be determined if unionoids in US waters have reached a critical, low threshold glycogen value that results in mortality.

Concentrations of total lipid and of individual fatty acids in L. siliquoides were not significantly correlated with zebra mussel infestation, but fatty acids do provide a possible explanation for differences in glycogen concentrations on either side of the Canada-US border. Unionoids from sites in US waters had higher levels of palmitoleic acid (16:1n-7) and eicosapentaenoic acid (20:5n-3) in their tissues, suggesting that they consume a diet rich in diatoms. Unionoids collected from Chematogan Bay on the Canadian side of the US border. Unionoids from sites in US waters had higher levels of palmitoleic acid (16:1n-7) and eicosapentaenoic acid (20:5n-3) in their tissues, suggesting that they consume a diet rich in diatoms. Unionoids collected from Chematogan Bay on the Canadian side of the US border. Unionoids from sites in US waters had higher levels of palmitoleic acid (16:1n-7) and eicosapentaenoic acid (20:5n-3) in their tissues, suggesting that they consume a diet rich in diatoms. However, due to the lack of definitive physiological thresholds, it cannot be determined if unionoids in US waters have reached a critical, low threshold glycogen value that results in mortality.
The unionid communities of the Lake St. Clair delta appear relatively stable over the 6 year interval since Zanatta et al.’s (2002) study. However, 6 years is a very short time interval for an animal that may live for several decades, so continued sampling of this potentially important refuge is needed to evaluate long term trends in population dynamics. The dominant offshore currents in the delta may prevent planktonic zebra mussel veligers from achieving high densities in shallow waters and this may be a factor limiting zebra mussel infestation of unionids in the delta. Even at the low rates of infestation observed, zebra mussels appear to be adversely affecting energy stores (as glycogen) in unionids from Canadian waters of the delta. The lack of a trend in concentrations of glycogen in unionids from US waters over the same gradient of infestation as seen in Canadian waters suggests that additional factors are affecting energy storage in unionids in the delta. Fatty acids in unionids indicate that there may be important differences in food quantity in the US vs. Canadian waters. This seems plausible since the source waters for the US side of the delta stem immediately from the more oligotrophic and diatom-rich waters of Lake Huron.

The goal of this study was to characterize the refuge for native freshwater mussels in the Lake St. Clair delta and not to make direct comparisons between US and Canadian waters. However, it is interesting to note that in almost every measure, whether it be unionid density, species richness, maximum shell length, or the concentration of glycogen and fatty acid composition of unionid tissues, there were differences between US and Canadian sites. Reasons presented here to explain differences in measures of physiological condition are speculative and more in-depth studies of the inter-relationships between glycogen and fatty acid concentrations in unionids, zebra mussels, and food type/availability are required.

With an area of ~100 km², the refuge in the delta of Lake St. Clair is 30 to 100× larger than the other known Great Lakes refugia, all of which are in Lake Erie. Based on the large size of this refuge, it is apparent that the delta supports the largest and richest unionid community remaining in the lower Great Lakes. The refuge also supports several species that are listed as Endangered and Threatened in Canada and/or Michigan, including significant populations of P. sinitoxia, Obluvoria subrotunda (Rafinesque, 1820), and L. nasuta. The populations of these species are likely the largest remaining in Canada (COSEWIC, 2003, 2004, and 2007b). Conserving the unionid communities of the Lake St. Clair delta will be critical to efforts aimed at preserving the highly imperiled unionid fauna of the Great Lakes.

Acknowledgments

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References


