

1 **Genetic structure of the benthic amphipod *Diporeia* (Amphipoda: Pontoporeiidae) and its**
2 **relationship to abundance in Lake Superior**

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21 **Abstract**

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23 The freshwater amphipod *Diporeia* is a crucial part of the food web in the Laurentian Great
24 Lakes, but has faced serious declines correlated with the invasion of zebra mussels (*Dreissena*
25 *polymorpha*), except in Lake Superior, which has seen an increase in *Diporeia* abundance.
26 Speculation on the mechanisms causing changes in *Diporeia* densities has not considered the
27 possibility of evolutionarily distinct lineages of *Diporeia* within the Lakes. In this study, we use
28 COI DNA sequence data to investigate the evolutionary history of Lake Superior *Diporeia*
29 relative to the other Great Lakes, and consider potential population structuring within Lake
30 Superior based upon depth or geography. Our analyses reveal that Lake Superior *Diporeia*
31 represent a distinct lineage that diverged from populations of the other lakes at least several
32 hundred thousand years ago. F-statistics show that two localities within Lake Superior were
33 significantly differentiated from all other locales, but analysis of molecular variance did not find
34 significant structure based on depth or geography. Genetic diversity within Lake Superior was
35 not correlated with depth, although abundance was significantly negatively correlated with
36 increasing depth.

37 **Keywords:** *Diporeia*, Great Lakes, zebra mussels, amphipod

38 **Introduction**

39 The amphipod genus *Diporeia* Bousfield is restricted to deep, glacial relict lakes in
40 northern North America (Bousfield 1989). In the Laurentian Great Lakes, *Diporeia* (6-9 mm
41 long as adults) historically has accounted for 60-80% of the benthic biomass (Dermott et al.
42 2005). Since the 1990s, *Diporeia* has been in serious decline in the Great Lakes, being virtually
43 extirpated from Lake Erie (Dermott and Kerec 1997), and declining drastically in Lakes
44 Michigan (Nalepa et al. 2006a,b), Ontario (Lozano et al. 2001, Lozano and Scharold 2005,
45 Watkins et al. 2007), and Huron (Nalepa et al. 2003). The reduced abundance is strongly
46 correlated with the invasion first by the zebra mussel (*Dreissena polymorpha*) (Ward and
47 Ricciardi 2007) and now by the quagga mussel (*D. bugensis*) (Watkins et al. 2007).

48 *Diporeia* have high lipid content (Cavaletto et al. 1996) and consequently are a very
49 important food source for many fishes of the Great Lakes (Pothoven et al. 2001, Pothoven and
50 Vanderploeg 2004). The decline of *Diporeia* has begun to impact several fishes in the Great
51 Lakes, including both small prey and larger species that are important commercially and
52 recreationally. Alewife (*Alosa pseudoharengus*), bloater (*Coregonus hoyi*), and slimy sculpin
53 (*Cottus cognatus*) have shifted their diets away from *Diporeia* to other benthos, and the density
54 of these fishes in the Great Lakes seems to be decreasing (Hondorp et al. 2005). Alewife also
55 have exhibited a decrease in weight (Madenjian et al. 2003, Pothoven and Madenjian 2008) and
56 energy density (Madenjian et al. 2006) which in turn may negatively affect the growth of
57 Chinook salmon (*Oncorhynchus tshawytscha*) (Madenjian et al. 2006), a popular sport fish and
58 important predator of alewife. Lake whitefish (*Coregonus clupeaformis*) have shown a decrease
59 in the amount of *Diporeia* in their diets (70% down to 25%), which has led to detrimental
60 changes in the growth patterns of this species, increased age when reaching sexual maturity

61 (Pothoven et al. 2001, Pothoven and Madenjian 2008) and reduced egg production (Kratzer et al.
62 2007). Commercial harvest of whitefish in Lake Ontario declined from 295,000 kg in 1996 to
63 100,000 kg in 2001 (Hoyle 2005).

64 The *Diporeia* in Lake Superior have not experienced a similar decline, and recent studies
65 have shown that densities there have not decreased substantially since the 1970s (Auer and Kahn
66 2004, Scharold et al. 2004, 2008). Lake Superior *Diporeia* are found at increased densities in
67 near-shore environments (30-70 meters deep) (Scharold et al. 2004) in contrast with the
68 distribution of remaining populations in the other Great Lakes where *Diporeia* are relegated to
69 deeper refuges (Watkins et al. 2007). *Dreissena* had invaded Lake Superior by 1989 (O'Neill and
70 Dextrase 1994) but are restricted to a few bays, possibly due to the physical and chemical
71 characteristics of the lake that may have inhibited the expansion of dreissenids (Grigorovich et
72 al. 2003). The *Diporeia* populations in Lake Superior therefore have not experienced the
73 widespread encroachment of *Dreissena* into their habitats as in the other Lakes.

74 All the hypotheses put forward to explain the losses of *Diporeia* from the lower Great
75 Lakes concurrent with the lack of decline in Lake Superior *Diporeia* rely on external influences
76 (Nalepa et al. 2006a,b), and have thus far neglected the possibility that there may be significant
77 evolutionary and, thus, ecological differences between distinct populations of *Diporeia* in the
78 Great Lakes. Unfortunately, the taxonomy of the genus *Diporeia* currently is not well
79 understood. *Diporeia hoyi* is considered to be the dominant species in the Great Lakes, but four
80 other species of *Diporeia* (including two that are undescribed) have been reported to occur in the
81 region (Bousfield 1989). Species in the genus can be difficult to identify as females dominate the
82 life cycle and are collected predominantly instead of more morphologically distinct males
83 (Bousfield 1989). Given the possibility of evolutionarily distinct lineages of *Diporeia*, variations

84 in declines may represent differential ecological responses to recent stressors by genetically
85 divergent populations. The clear differences between current patterns of abundance in the Great
86 Lakes may reflect underlying taxonomic differences between *Diporeia* populations.

87 The aim of this study was to test the null hypothesis that *Diporeia* in the Great Lakes
88 represent a single evolutionary lineage. Using DNA sequence data from the mitochondrial
89 cytochrome c oxidase subunit I (COI), we assess the degree of genetic divergence between
90 *Diporeia* populations in Lake Superior and those in Lakes Huron, Michigan, and Ontario. We
91 also explore in more detail the patterns of genetic variation found in Lake Superior by testing
92 hypotheses regarding the structuring of genetic variation by geography and by depth, and by
93 assessing relationships between genetic diversity and observed patterns of abundance. These
94 results reveal fundamental differences between *Diporeia* in Lake Superior and populations
95 remaining in the other Lakes; although the differences do not explain declines in other Lakes, the
96 data provide important new background context on *Diporeia* distribution in and across Lakes.

97 **Materials and methods**

98 **Sample Collection**

99 Specimens of *Diporeia* were collected by multiple ponar grabs at each locality in August
100 2007. Depth and GPS coordinates were recorded for each collection site. Five of the collection
101 sites came from near shore localities with depths less than 70 m (SN01, SN17, SU04, SU06, and
102 SU22B), and three sites came from deeper, off-shore localities deeper than 150 m (SU10, SU11,
103 SU19) (Table 1). Abundances in number of individuals per square meter were calculated for
104 each collection site. The specimens were removed from the sediments and stored in 95% EtOH
105 for later use in the molecular study. To compare Lake Superior samples to the other lakes,

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106 samples of *Diporeia* were collected at one site in Lake Huron, two sites in Lake Michigan, and
107 two sites in Lake Ontario (Table 1).

108 **Molecular study**

109 DNA extractions were done with one half to whole vacuum-dried specimens dependent
110 on size of the individual. All specimens were extracted using the DNeasy Tissue Kit from
111 QIAgen following the manufacturer's protocol. PCR amplification of a 658 bp fragment of the
112 mitochondrial cytochrome c oxidase subunit I gene (COI) was done in a 20 μ L volume reaction
113 under the following conditions: standard buffer concentration, 2.25 mM MgCl₂, 200 pM dNTPs,
114 0.25 μ M of each primer (standard DNA barcoding primers LCO-1490F and HCO-2198R
115 (Folmer et al. 1994)), ½ unit of QIAgen Taq polymerase, 400 ng BSA, and approximately 20 ng
116 of template DNA. The PCR amplification program was an initial step of 94°C for 150 sec, 35
117 cycles of 94°C for 30 sec, 46°C for 60 sec, and 72°C for 60 sec, and a final step of 72°C for 10
118 min. Additional PCR of the internal transcribed spacer regions (ITS1 and ITS2) was performed
119 for 8 individuals (4 from Lake Michigan and 4 from Lake Superior with the primers gc18SF (5'-
120 GGCGTCGTCGTGCTCG-3') and gc28SR (5'-CCTCACCCACCTAGTAG-3') following the
121 above conditions and program. PCR products were cleaned using the QIAquick PCR kit on a
122 QIAgen BioRobot 3000. Sequencing reactions were done with the ABI Big Dye Terminator
123 Cycle Sequencing Ready Reaction kit following the manufacturer's protocol. The sequenced
124 products then were purified using the DyeEx 96 Kit from QIAgen, dried, and re-eluted with
125 formamide, and then run on an ABI Prism 3730xl DNA Analyzer. All products were sequenced
126 in both directions and were compiled into single contiguous sequences with Sequencher 4.8
127 (Gene Codes, Ann Arbor, MI). All sequences have been deposited in GenBank (accession
128 numbers COI: EU761246 to EU761577; ITS: EU807701 to EU807708).

129 After alignment of the COI exports in Sequencher, the data set was analyzed using
130 MEGA 3.1 (Kumar et al. 2004) to determine genetic distances (K2P model for corrected
131 distances) and conduct neighbor-joining (NJ) cluster analysis, and Nona (v 2)/Winclada (v
132 0.9.99) (Goloboff 1999, Nixon 1999) were used for a maximum parsimony analysis with 1000
133 bootstrap pseudoreplicates. A minimum spanning network was generated using Network 4.5
134 (Fluxus Technology, Suffolk, England). The data set also was analyzed in Arlequin 3.11
135 (Excoffier et al. 2005) to generate haplotype diversity, nucleotide diversity, and fixation index
136 (F_{st}) values. Analyses of molecular variance (AMOVA) were done in Arlequin to determine if
137 either depth or location had an influence on population structure. To test for partitioning of
138 genetic variance by depth, one group was defined containing all populations found at depths less
139 than 100 m (near-shore: SN01, SN17, SU04, SU06, SU22B) and a second including those found
140 at depths greater than 100 m (off-shore: SU10, SU11, SU19). To test for geographic structure,
141 the populations were placed in eastern (SU04, SU06, SU10, SU11) or western (SN01, SN17,
142 SU19, SU22B) groups based on their location relative to Keweenaw Point. To test for recent
143 demographic expansion in the Lake Superior population we generated a distribution of the
144 frequency of pairwise number of nucleotide mismatches between all Lake Superior COI
145 haplotypes using Arlequin. Rapid population expansion results in the accumulation of mutations
146 with minimal loss of lineages, resulting in a distinctive unimodal peak in this mismatch
147 distribution. Steady-state populations, in contrast, exhibit multimodal or “ragged” distributions
148 reflecting equilibrium between mutation accumulation and stochastic loss of lineages. The
149 statistical significance of the observed distribution’s departure from the expectation of
150 unimodality (the “raggedness index”) can be assessed by simulation to test the hypothesis of
151 recent demographic expansion. In addition, since accumulation of mutations increases the mean

152 of the mismatch distribution (τ), that value can be used to estimate the time in generations
153 since population expansion, given a known per-sequence mutation rate (Rogers and Harpending,
154 1992, Excoffier et al. 2005). To visually assess genetic relationships between Superior
155 populations, we constructed a multi-dimensional scaling plot of the F_{st} values using SAS (9.1.3).

156 **Results**

157 We were able to successfully sequence a 658 base pair (bp) fragment of COI for 235
158 individuals from Lakes Superior, 74 from Lake Michigan, 13 from Lake Ontario, and 10 from
159 Lake Huron. Within Lake Superior we found 59 different haplotypes, and within the other three
160 Great Lakes we found 37 haplotypes. Multiple haplotypes were found at each locality, ranging
161 from 3 to 16 different haplotypes at the Lake Superior sites, and from 4 to 15 at the sites in Lakes
162 Huron, Michigan, and Ontario (Table 1). In Lake Superior haplotype diversity (H_e) ranged from
163 0.4571 (site SU10) to 0.9048 (site SU22B) with an overall haplotype diversity of 0.8447 for the
164 entire lake (Table 1). The near-shore, shallow sites had higher average haplotype diversity (mean
165 = 0.7842) than the off-shore, deep sites (mean = 0.6146), but a t-test of the data did not show
166 these differences to be significant ($P = 0.157$). An analysis of correlation between H_e and
167 abundance was not significant ($P = 0.138$), however, a strong negative correlation between
168 abundance and depth was found ($P = 0.009$). For Lakes Huron, Michigan, and Ontario haplotype
169 diversity ranged from 0.7500 to 1.0 with an overall diversity of 0.9156, which did not differ
170 significantly from that found in Lake Superior (t-test $P = 0.0567$). Nucleotide diversity ranged
171 from 0.000753 to 0.003860 (mean = 0.002417) in Lake Superior and from 0.001411 to 0.006293
172 (mean = 0.003773) in the other Great Lakes, and a t-test did not find the means significantly
173 different ($P = 0.184$).

174 None of the COI haplotypes found in Lake Superior were found in the other Great Lakes
175 (Fig.1). Both cluster (NJ) and maximum parsimony analysis of the COI haplotypes across the
176 four Great Lakes revealed a distinct separation between the Lake Superior haplotypes and those
177 of the other Great Lakes with high bootstrap support (100% and 97%, respectively; trees not
178 shown). Six fixed point differences were found between the Lake Superior haplotypes and the
179 haplotypes of the other lakes (Table 2). The mean genetic distance observed between individuals
180 in Lake Superior was 0.31% (range: 0 to 1.54%). Genetic distances between individuals of Lake
181 Superior and individuals from the other Great Lakes had a mean of 1.69% (range: 0.92 to
182 2.64%).

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183 Amplification of the ITS regions was successful for all 8 specimens attempted. The ITS1
184 was relatively short at 284 bp, but the ITS2 was considerably longer at an estimated 946 bp.
185 Seven of the individuals (3 from Lake Michigan and 4 from Lake Superior) had identical
186 sequences for both loci. The other individual from Lake Michigan differed by only a single base
187 pair in the ITS2 and its ITS1 sequence was identical to the other 7 specimens.

188 The most common COI haplotype (SUP h01) in Lake Superior was found at all 8 sites
189 and occurred in 85 (36.0%) individuals. This haplotype was rare at some sites (e.g. 11.1% of the
190 population at SU19) and common at others (e.g. 73.3% at SU10). Of the 59 COI haplotypes
191 found in Lake Superior, only 10 haplotypes were shared between multiple sites (Fig. 2). The
192 other 49 haplotypes were unique to a given locality with 38 of these haplotypes found only in
193 single individuals. The second most common haplotype (SUP h02—34 individuals) was found in
194 individuals at SN01, SN17, SU19, and SU22B, all western sites. Most of the haplotypes (46 out
195 of 58) differ from SUP h01 by 1 or 2 bp, and the overall average genetic distance between Lake

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196 Superior haplotypes was 0.58%. Mismatch distribution analysis (Fig. 3) of all Lake Superior
197 individuals revealed a value of 1.184 for τ , and a raggedness index of 0.028 ($P = 0.99$).

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198 A comparison of F_{st} values between localities suggested that some population structure
199 may be present in Lake Superior. Two western, near-shore populations (SN17 and SU22B) were
200 significantly differentiated from all the eastern populations and also from SU19, the only
201 western, off-shore population (Table 3). The populations SU04 (eastern, near-shore) and SU19
202 (western, off-shore) had significant F_{st} values when compared against all other populations. A
203 multi-dimensional scaling plot incorporating all F_{st} values between populations shows
204 populations SU04 and SU19 as potential outliers to a cluster of the remaining populations (Fig.
205 4). Despite AMOVA results indicating significant genetic differentiation between individual
206 collection sites (consistent with F_{ST} estimates), overall differentiation between western and
207 eastern regions of Lake Superior was not significant ($P = 0.0704$). Similarly, we found no
208 evidence to support the hypothesis of genetic differentiation between near- and off-shore sites in
209 Superior (AMOVA partitioned by depth, $P = 0.462$) (Table 4).

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210 Discussion

211 The lack of shared COI haplotypes between populations of *Diporeia* in Lake Superior
212 and populations in Lakes Huron, Michigan, and Ontario renders these populations reciprocally
213 monophyletic and strongly suggests absence of significant gene flow between *Diporeia* of Lake
214 Superior and the other Great Lakes. Although the *Diporeia* in Lake Superior appear to have
215 diverged evolutionarily from populations in the other Great Lakes, our data are currently not
216 strong enough to support consideration of the Lake Superior *Diporeia* as a distinct species. The
217 minimum interpopulation genetic distance is only 0.92% for COI, which is considerably smaller
218 than the maximum intrapopulation variation found in Lake Superior (1.54%). Furthermore, the

219 identical sequences of the ITS1 and ITS2 regions (two loci that are often considered to show
220 species-specific differences (Pilgrim and Pitts 2006, Pilgrim and von Dohlen 2007)) that occur in
221 the different lake populations are also suggestive that the *Diporeia* in all the Great Lakes have
222 not diverged enough to show differences in these nuclear loci. More extensive sampling of
223 individuals from Lakes Huron, Michigan, and Ontario would be necessary to resolve whether
224 these lineages constitute separate species. Our current sampling is not consistent, however, with
225 the hypothesis that as many as four different *Diporeia* species (Bousfield 1989) occur in these
226 lakes.

227 Whether treated as a single or separate species, the genetic distance between the
228 populations of Lake Superior and the other lakes suggests significant evolutionary divergence
229 between the two lineages. An estimate of time of divergence based on a widely utilized COI
230 mutation rate (1.4%/MYA) in crustaceans (Knowlton and Weigt 1998) using the minimum
231 distance between haplotypes (0.92%, representative of the six fixed mutational differences
232 between lineages) places the split between Lake Superior *Diporeia* and the other Great Lakes
233 lineage at least 650,000 years ago (Pleistocene). Using more conservative COI mutation rates
234 (0.19% to 0.55%/MYA) advocated by other authors (Schön et al. 1998, de Bruyn 2005) places
235 the divergence at 1.67 MYA (Pleistocene) to 4.84 MYA (Pliocene). Although some consider
236 dating nodes based on estimated mutations rates rather than fossil evidence to be problematic
237 (Heads 2005), all the estimates here place the split between Lake Superior *Diporeia* and the other
238 Great Lakes at least several hundred thousand years before the most recent formation of the
239 Laurentian Great Lakes (10,000-15,000 years ago). More concrete estimates of the divergence
240 between the Great Lakes populations would be better addressed with a phylogeny of the entire
241 genus. The unimodal mismatch distribution (Fig. 3) for the Lake Superior lineage is indicative of

242 a rapid demographic expansion in the *Diporeia* lineage currently inhabiting that lake, and using
243 the estimates of mutation rate for COI above, this expansion can be placed between 400,000 and
244 3.1 million years ago. These estimates are consistent with a scenario of rapid population
245 expansion in the Lake Superior lineage following evolutionary divergence from the lineage
246 founding populations in Huron, Michigan, and Ontario, with both events greatly pre-dating the
247 formation of the Laurentian Great Lakes. These estimated divergence times also are comparable
248 to divergence estimates found in freshwater fishes such as white sucker (*Catostomus*
249 *commersoni*) (Lafontaine and Dodson 1997), brown bullhead (*Ameiurus nebulosus*) (Murdoch
250 and Hebert 1997), lake trout (*Salvelinus namaycush*) (Wilson and Hebert 1998), and banded
251 killifish (*Fundulus diaphanus*) (April and Turgeon 2006), all of which have had their
252 evolutionary histories and geographic distributions influenced by past North American glacial
253 events.

254 The segregation of the Lake Superior *Diporeia* from the populations of the other Great
255 Lakes is consistent with population studies of fish species distributed throughout the lakes. The
256 walleye (*Stizostedion vitreum*) of Lake Superior were distinct from lakes Michigan, St. Clair,
257 Erie, and Ontario based on mtDNA control region haplotypes (Stepien and Faber 1998).
258 Microsatellite data show that populations of smallmouth bass (*Micropterus dolomieu*) in Lake
259 Superior have not experienced gene flow with the other Great Lakes (Stepien et al. 2007). The
260 distinctness of the Lake Superior populations of smallmouth bass and walleye are also congruent
261 with patterns found in yellow perch (*Perca flavescens*) and brown bullhead (*Ameiurus*
262 *nebulosus*) (Stepien et al. 2007). The dispersal capabilities of these fish species very likely is
263 much greater than that of *Diporeia*, and therefore the lack of gene flow in this amphipod between
264 Lake Superior and the other lakes should be expected.

265 Although the abundance of *Diporeia* outside Lake Superior has declined drastically, the
266 comparable haplotype diversity between Lake Superior and the other Great Lakes (except for
267 Lake Erie where *Diporeia* have been virtually extirpated) suggests that the decline has not
268 resulted in a genetic bottleneck for the remaining populations. Within Lake Superior, the near-
269 shore localities exhibit higher genetic diversity based both on a higher percentage of individuals
270 with unique haplotypes (22.5% to 16.1%; see fig. 2) and on overall haplotype diversity (0.7842
271 vs. 0.6146), but these differences in diversity were not significant. The widely disparate
272 abundances in *Diporeia* seen here (Table 1) and in previous studies (Scharold et al. 2004, 2008)
273 between near-shore and off-shore collection sites originally led us to investigate genetic
274 population structure in the lake. No significant population structure was found between shallow
275 and deep collection sites and this suggests that gene flow regularly occurs between near-shore
276 and off-shore habitats. Comparisons of eastern vs. western collection localities also did not show
277 significant structure. An eastern, near-shore population (SU04) and a western, off-shore
278 population (SU19) were both shown to be significantly different from all other populations based
279 on F_{st} values, implying that some population structuring is present in the lake. Although these
280 two populations are outliers, they do suggest that population structuring exists in *Diporeia* of
281 Lake Superior, but that neither of the broad hypotheses tested here (east vs. west or near-shore
282 vs. off-shore) is a sufficient explanation for that structure. Dispersal in *Diporeia*, however, is
283 male biased (Bousfield 1989), and as a consequence, the maternally inherited COI haplotypes
284 may underestimate gene flow among populations within the lake. More comprehensive sampling
285 and the use of nuclear markers may provide a more complete picture of genetic structure within
286 Lake Superior.

287 The populations of *Diporeia* in Lake Superior, especially the near-shore habitats, have
288 not suffered a decline correlated with zebra mussel invasion (Scharold et al. 2004, 2008) as have
289 the *Diporeia* of the other Laurentian Great Lakes. In fact, the abundance of *Diporeia* in Lake
290 Superior seems to have increased 5- to 8-fold over levels seen in the early 1970s (pre-*Dreissena*
291 invasion), possibly due to a decrease in pollution and the rebound of the lake trout, which feeds
292 on the predators of *Diporeia* (Scharold et al. 2004, 2008). The data in this study show that
293 *Diporeia* of Lake Superior have diverged from the populations of the other lakes at least several
294 hundred thousand years ago, and the distinct evolutionary history of Lake Superior *Diporeia*
295 should be taken into account in any work that seeks to explain the current status of *Diporeia*
296 within Lake Superior. That the zebra mussel occurs in Lake Superior is not in question, but it
297 does not appear to be fully established in the lake (O'Neill and Dextrase 1994, Grigorovich et al.
298 2003, Scharold et al. 2004) because known populations are not consistently found year-to-year
299 (Grigorovich et al. 2003), possibly due to Lake Superior's physical and chemical characteristics
300 such as depth, temperature, and nutrient content which may approach the habitat limits of zebra
301 mussels (Grigorovich et al. 2003). Certainly, the divergent evolutionary history of the Lake
302 Superior *Diporeia* could be one factor in the current high population abundances in Lake
303 Superior, but to assume that the genetic differences are the only explanation is not prudent,
304 especially considering the invasion of Lake Superior by zebra mussels has not been as severe as
305 in the other Great Lakes. This is further emphasized by the fact that in the Finger Lakes of New
306 York, *Diporeia* populations are not declining despite the presence of high densities of dreissenids
307 (Nalepa et al. 2006b), although they belong to the same genetic lineage as the *Diporeia* found in
308 Huron, Michigan and Ontario (data not shown). The Lake Superior populations and the lower
309 Great Lakes populations each could be considered unique evolutionary lineages warranting

310 management as the loss of *Diporeia* in Lakes Huron, Michigan, and Ontario would not be
311 mitigated by the lack of decline in Lake Superior *Diporeia*. The *Diporeia* of Lake Superior may
312 face new challenges in the recent introduction of the quagga mussel (*D. bugensis*) (Vanderploeg
313 et al. 2002, Grigorovich et al. 2008), or by expansions in the ranges of invasive fish such as ruffe
314 (*Gymnocephalus crenuus*) (Bauer et al. 2007) or the round goby (*Neogobius melanostomus*).

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Table 1. Locality, abundance information, and genetic diversity statistics per site.

Site	Locality	Depth	Abundance (m ⁻²)	No. Individuals	No. Haplotypes	Haplotype diversity	Nucleotide diversity
<i>Lake Superior</i>							
SN01	46.75378N 91.91447W	31m		32	13	0.8085 ± 0.0605	0.002831 ± 0.001852
SN17	46.79446N 91.56222W	32m	3569	62	16	0.8112 ± 0.0345	0.002854 ± 0.001834
SU04	46.52617N 84.83017W	69m	1449	28	8	0.7487 ± 0.0702	0.003349 ± 0.002124
SU06	47.10950N 88.21517W	68m	2254	23	10	0.6403 ± 0.1165	0.001694 ± 0.001279
SU10	47.51416N 87.54617W	157m	2043	15	3	0.4571 ± 0.1406	0.000753 ± 0.000763
SU11	47.37033N 85.96500W	208m	13	29	9	0.7266 ± 0.0859	0.002313 ± 0.001592
SU19	47.13333N 90.85400W	191m	290	18	4	0.6601 ± 0.0781	0.001679 ± 0.001287
SU22B	46.80000N 91.74967W	55m	51	28	14	0.9048 ± 0.0341	0.003860 ± 0.002381
1785							
<i>Lake Huron</i>							
HU96B	44.58317N 81.50000W	49m	n/a	10	8	0.9556 ± 0.0594	0.004712 ± 0.003038
<i>Lake Michigan</i>							
MI11	42.38267N 87.00017W	130m	n/a	43	15	0.7553 ± 0.0683	0.003272 ± 0.002057
MI40	44.75984N 86.96684W	173m	n/a	31	14	0.8968 ± 0.0322	0.003177 ± 0.002029
<i>Lake Ontario</i>							
ON41	43.71667N 78.02700W	135m	n/a	8	4	0.7500 ± 0.1391	0.001411 ± 0.001230
ON55M	43.44333N 77.43834W	201m	n/a	5	5	1.0000 ± 0.1265	0.006293 ± 0.004457

Table 2. Fixed differences in COI haplotypes

between Lake Superior and the other Great Lakes. Numbers are relative to the start of the COI fragment analyzed in the current study.

	202	265	421	458	496	589
Superior	A	A	A	G	A	C
other Great Lakes	T	G	C	A	G	T

Table 3. Pairwise F_{st} values between sites in Lake Superior, with statistical support. Significant differences are shown in bold.

		SN01	SN17	SU04	SU06	SU10	SU11	SU19
SN17	F_{st}	0.00510						
	P-value	0.28829						
SU04	F_{st}	0.17296	0.18152					
	P-value	<<0.0001	<<0.0001					
SU06	F_{st}	0.03561	0.10025	0.23498				
	P-value	0.05405	<<0.0001	<<0.0001				
SU10	F_{st}	0.08256	0.15052	0.30710	0.00474			
	P-value	0.05405	<<0.0001	<<0.0001	0.27027			
SU11	F_{st}	0.02804	0.07905	0.19823	0.00189	0.03013		
	P-value	0.05405	<<0.0001	<<0.0001	0.30631	0.10811		
SU19	F_{st}	0.17127	0.13849	0.28071	0.30311	0.38689	0.26154	
	P-value	<<0.0001	<<0.0001	<<0.0001	<<0.0001	<<0.0001	<<0.0001	
SU22B	F_{st}	0.02384	0.03517	0.14717	0.10882	0.17073	0.08291	0.16106
	P-value	0.09009	0.05405	<<0.0001	0.00901	<<0.0001	<<0.0001	<<0.0001

Table 4. AMOVA results of Lake Superior populations.

	Eastern vs. Western		
	Variance Components	Percentage of Variation	Fixation Indices
<i>Among Groups</i>	<i>0.01798</i>	<i>4.11</i>	<i>0.0411</i>
Among Populations within Groups	0.04261	9.73	0.1015*
Within Populations	0.37719	86.16	0.1384*
	Near Shore vs. Off Shore		
	Variance Components	Percentage of Variation	Fixation Indices
<i>Among Groups</i>	<i>-0.00447</i>	<i>-1.05</i>	<i>-0.0105</i>
Among Populations within Groups	0.05486	12.83	0.1270*
Within Populations	0.37719	88.21	0.1179*

*P-value < 0.05

Figure Captions:

Fig. 1. Minimum-spanning network of *Diporeia* COI haplotypes and their North American Great Lakes of origin (**E**: Lake Erie; **H**: Lake Huron; **M**: Lake Michigan; **O**: Lake Ontario; **S**: Lake Superior). The top left boxed cluster is a group of haplotypes found only in Lake Superior. The top right boxed cluster is a group of haplotypes found in Lakes Huron, Michigan, and Ontario. The size of each circle is proportional to the number of individuals that had that haplotype. Un-sampled/missing intermediate haplotypes are marked with black squares. The colored portions of each circle in the network correspond to the colored box of each locality. The thicker black line between the two networks highlights the six fixed base pair differences between the *Diporeia* haplotypes of Lake Superior and those of Lakes Huron, Michigan, and Ontario.

Fig. 2. Haplotype distribution among populations in Lake Superior. The dark blue areas of each pie chart denote the proportion of the most common haplotype at each locality. The white areas of the pie charts denote haplotypes that are unique to that population. The other color patterns show haplotypes shared among at least two populations.

Fig. 3. Observed mismatch distribution for Lake Superior COI haplotypes (columns) plotted with the simulated expectation (dotted line) based on the assumption of rapid demographic expansion followed by stable large population size. The raggedness statistic estimates departure of the observed distribution from the null model expectation, and its significance is indicated in the figure.

Fig. 4. Multi-dimensional scaling plot based on the F_{st} values between each population in Lake Superior. Gray square: eastern, near-shore; black square: eastern, off-shore; gray circle: western, near-shore; black circle: western, off-shore. Six of the localities form a cluster around the lower left quadrant. Population SU19 (a western, off-shore population) and Population SU04 (an eastern, near-shore population) are significantly different from all other populations based on F_{st} values.

Figure 1.

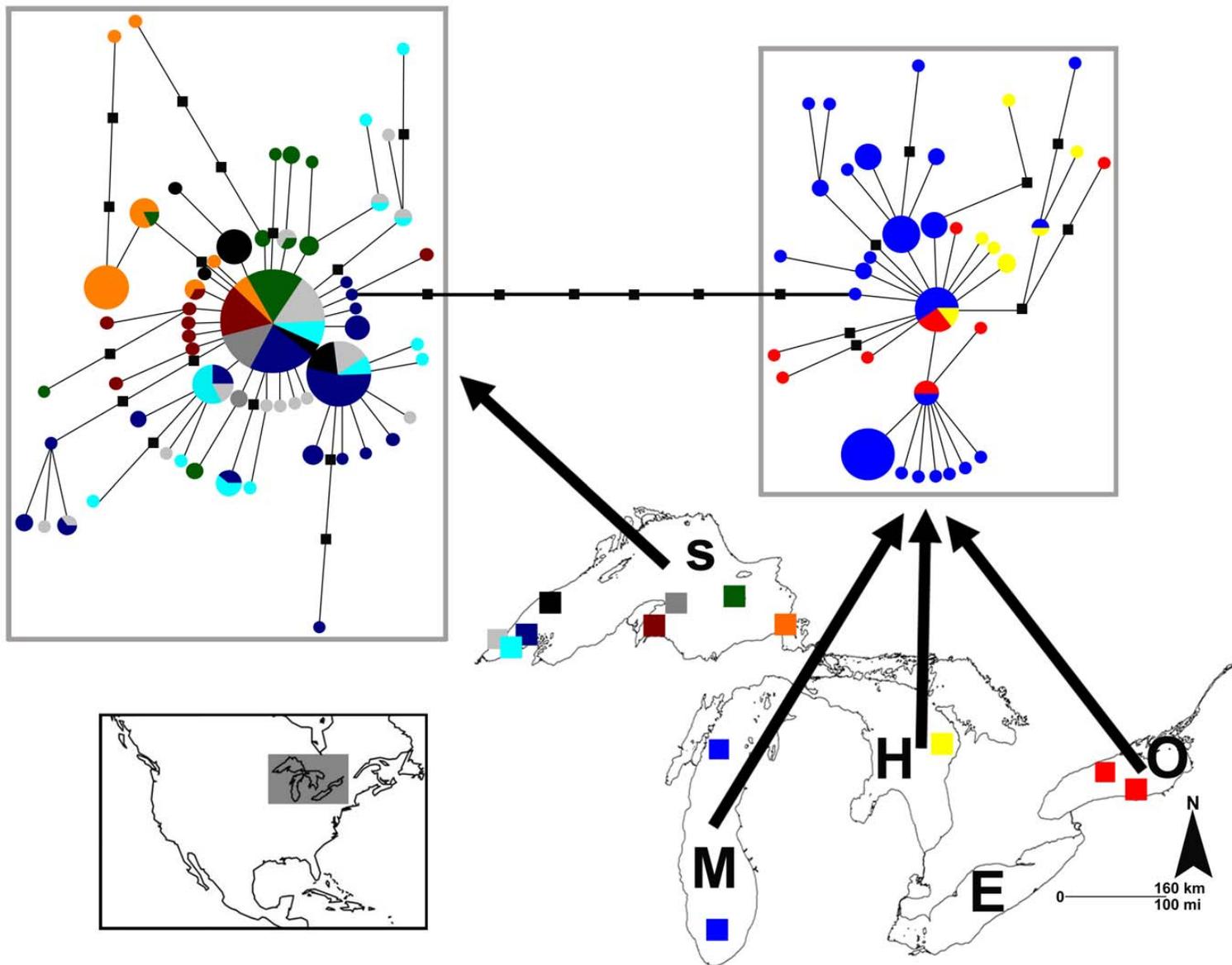


Figure 2.

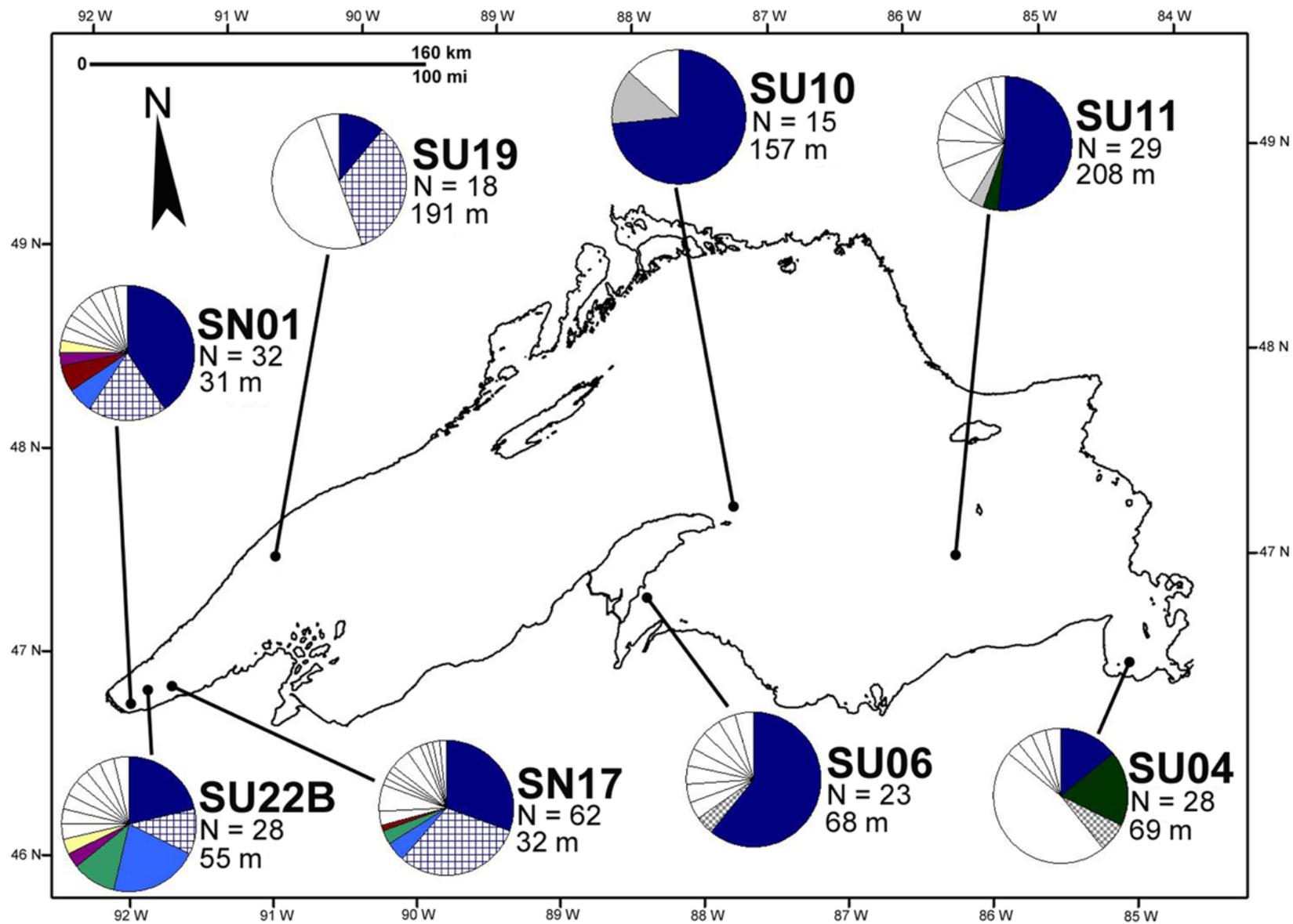


Figure 3.

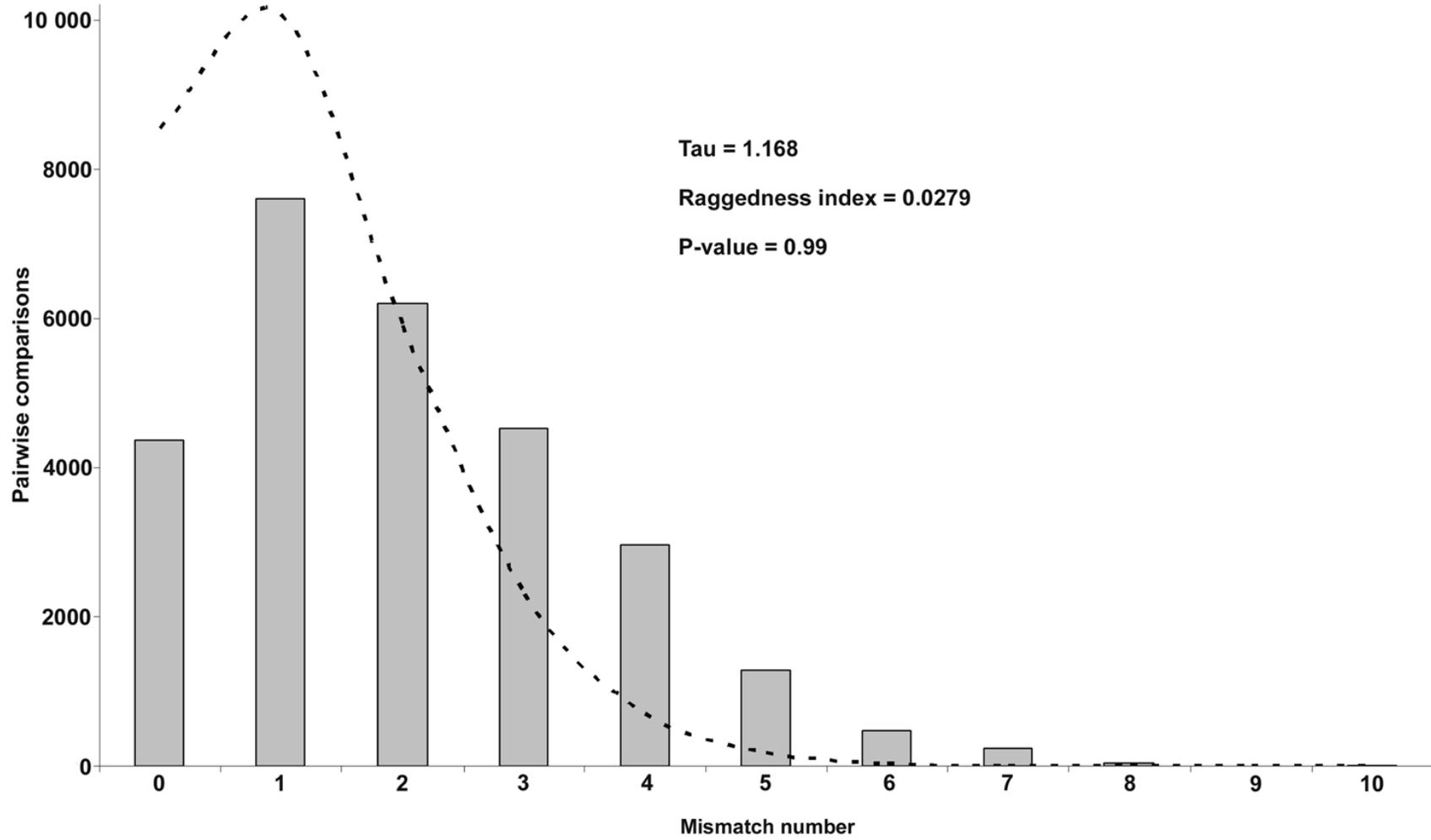


Figure 4.

