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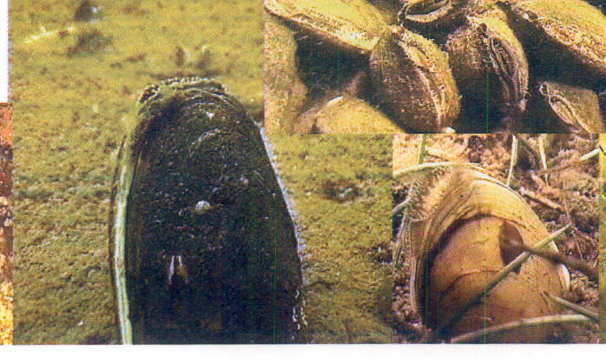


















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Status of Freshwater Unionid Populations at Isle Royale National Park, 1999-2001.

S. J. Nichols, E. Crawford, J. Amberg, J. Allen, G. Black,
and G. Kennedy
U.S. Geological Survey, Great Lakes Science Center
1451 Green Rd.
Ann Arbor, MI, 48105.

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And
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Corresponding author: Nichols
Email S_Jerrine_Nichols@ usgs.gov Phone- 734-214-7218

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INTRODUCTION

Unionid mussels (freshwater clams) are the most endangered group of animals in North American (Williams et al. 1993). North America has the largest diversity of unionids in the world (Metcalfe-Smith et al. 1997), and most of these are located in the Midwestern region of the United States. When compared to historical populations, many streams in eastern North America now possess depauperate mussel fauna. Williams et al. (1993) listed 297 species of native freshwater mussels in the United States and Canada. Of these, 213 species (71.7%) are considered endangered, threatened, or of special concern. Many of these species, 51 in the United States, are listed as endangered, and more are under review.

Unionid populations are declining due to a number of factors relating to habitat alteration and human interference. Problems stem from changes in physical habitat such as increased siltation, sedimentation and channelization; changes in water quality due to increased pollution such as heavy metals, radionucleides, pesticides, human and feed lot wastes, mining wastes, acid runoff; and harvesting for shell and pearls (e.g. Fuller 1974; Havlik and Marking 1987; Turner and Rabalais 1991; Schloesser et al. 1996,). The increased spread of exotic species (i.e., the zebra mussel), have placed additional stress on fragile populations, causing major extirpations of all unionid species in many regions (e.g. Schloesser and Nalepa 1994; Strayer and Smith 1996; Strayer 1999).

Perturbations of communities have caused resource managers to recognize the need for a transition from management of individual species to community management approaches (Christie et al. 1987; Evans and Waring 1987; Steedman and Regier 1987). Holistic management of communities has been hampered by lack of information on community structure, which is particularly scarce for unionid mussels. Managing mussel communities in any habitat requires describing each community, defining objectives for the structure of each community, and developing a means of measuring progress toward achievement of these goals. The goal of this project is to determine the population structure (distribution and diversity) and current status of native unionid mussel species at a number of national parks along the Great Lakes, including Isle Royale National Park.

The objectives are:

1. What unionid and other easily identified groups of bivalves are present in representative lakes and streams at ISRO?

2. At these same sites, which species fall into quickly ascertainable age classifications (i.e., juvenile, adult) based on size? Which species are actively recruiting?

3. What is the overall status of the population- stable, marginal, or at-risk?

4. What are the key environmental variables at each habitat sampled and are specific unionid communities associated with certain variables?

5. What is the quantity of each species present based on randomized quadrats or transects?

6. What is the annual incremental increase in shell length, or growth rate, for each species?

7. What is the amount and type of chemical contaminant present per gm of soft body tissue for each species sampled?

8. Management, regulatory, or additional study decisions or potential actions that might hinge on the results of the study include deciding:
   1) if unionid and other bivalve populations in various ISRO lakes are in good shape, appear to be under stress, or are at risk based on current status.
   2) what type of long term monitoring of unionids and other bivalves is needed (if any) to keep an eye on trends.
   3) whether or not to try to eradicate or otherwise manage non-native bivalve species, hosts, or other biota that might be threatening native bivalve species.

STUDY AREA

Isle Royale National Park, Michigan, is a remote wilderness archipelago located in northwest Lake Superior, 24 km from Canada, and 80 km from the Kewenaw Peninsula of Michigan. The main island in this archipelago, Isle Royale, is 72 km long and 14 km wide. There are about 400 smaller surrounding islands. Isle Royale consists of a series of bedrock ridges and valleys interspersed with numerous lakes varying in size, depth, and elevation above Lake Superior.

Eleven inland lakes on the main island of Isle Royale and one Lake Superior cove were quantitatively sampled; two other lakes were only visually surveyed (Table 1). All types of inland lakes were represented in the quantitative sampling, including oligotrophic, cold water lakes (Desor, Siskiwit), shallow wetland lakes (Feldtmann), isolated perched lakes (Hatchet) plus some of the most heavily used lakes on the island (Chickenbone-Livermore-LeSage corridor).

Table 1. Bivalve distribution at the sites surveyed by scout team in Isle Royale National Park, 1999-2000.

SITE	UNIONIDS	SPHAERIDS[1]	SPONGES[2]	EXOTICS[3]
Chickenbone Lake	present	present	present	none
Desor Lake	present	absent	absent	none
Feldtmann Lake	absent	absent	absent	none
Hatchet Lake	absent	absent	absent	none
Intermediate Lake	present	present	present	none
"Leech" Lake	absent	absent	absent	none
Livermore Lake	present	present	present	none
LeSage Lake	present	present	present	none
McCargoe Cove	present	present	absent	none
Richie Lake	present	present	absent	none
Sargent Lake[4]	present	not determined	not determined	not determined
Siskiwit Lake	present	present	absent	none
Whittlesey Lake	present	absent	absent	none
Wood Lake	present	not determined	not determined	not determined

[1] Sphaerid (fingernail clams) presence or absence provided as reference only- no further identifications were made. [2] Refers to large sponge colonies. [3] Exotic bivalves = zebra mussels (*Dreissena polymorpha*), quagga mussels (*Dreissena bugensis*), or Asian clams (*Corbicula fluminea*). [4] Visual sampling for unionid presence or absence.

Water quality data and morphometric for most of these eleven lakes was obtained from Kallemeyn et al. (2000) and is provided in Tables 2 and 3. The one exception is the lake we have labeled as "Leech" Lake. This is an unnamed lake located on the Minong Ridge Trail (Figure 1) and was not sampled by Kallemeyn et al. (2000). Water quality data for the Lake Superior cove, McCargoe Cove, is also not available.

Table 2. Physical characteristics of lakes quantitatively sampled for unionids in Isle Royale National Park, Michigan, 2000-2001. Data source Kallemeyn et al. (2000).

Lake	Elevation (m)	Area (ha)	Watershed Area (ha)	Max. length (km)	Max. width (km)	Max. Depth (m)	Relative Depth (%)
Chickenbone Lake	1.2	92.6	1556.4	2.84	0.36	6.4	0.59
Desor Lake	260.3	427.8	1436.7	4.45	1.91	14.02	0.60
Feldtmann Lake	201.2	185.8	886.6	2.66	1.02	2.74	0.18
Hatchet Lake	229.9	49.6	502.2	1.90	0.41	5.20	0.65
Intermediate Lake	2.6.0	70.8	481.7	1.77	1.01	6.70	0.71
LeSage Lake	223.4	45.0	933.0	1.66	0.48	6.40	0.85
Livermore Lake	213.1	30.1	168.8	1.57	0.30	5.50	0.89
Richie Lake	191.4	216.2	2080.2	3.2	1.99	10.67	0.64
Siskiwit Lake	201.0	1635.2	7287.1	11.06	2.30	46.00	1.01
Whittlesey Lake	208.0	65.0	450.5	2.97	0.27	7.62	0.84

Table 3. Water chemistry, chlorophyll *a*, and transparency data for ten inland lakes in Isle Royale National Park, Michigan quantitatively sampled for unionids in 2000-2001. Data source Kallemeyn et al. (2000).

Parameter	Chickenbone	Desor	Feldtmann	Hatchet	Intermediate	LeSage	Livermore	Richie	Siskiwit	Whitefish
Chl *a* (mg/m^3)	2.00	3.8	0.27	3.62	3.35	3.19	1.48	2.86	1.07	2.51
TP (µ/L)	13	7	13	7	14	10	10	10	14	7
TN (µ/L)	0.54	0.61	0.49	0.41	0.47	0.34	0.32	0.45	0.27	0.25
NO_3N(µg/L)	16	13	<5	<5	<5	<5	5	<5	27	21
NH_4N (µg/L)	13	19	9	<10	<10	<10	<10	<10	14	<10
Ca (mg/L)	9.6	11.7	8.4	9.9	8.2	8.9	10.6	9.8	8.6	8.6
Mg (mg/L)	3.66	3.47	1.85	3.44	2.32	2.8	3.46	2.90	2.24	1.98
Na (mg/L)	2.08	1.83	1.08	1.75	1.08	1.39	2.09	1.49	1.16	1.18
K (mg/L)	0.39	0.72	0.20	0.54	0.76	0.53	0.42	ND	0.36	0.41
Al (µg/L)	10	10	26	10	16	11	9	36	5	13
SO_4 (mg/L)	2.94	2.55	3.18	2.29	2.95	2.84	3.00	3.24	4.49	2.66
SiO_2 (mg/L)	8.5	1.6	1.5	10.7	2.3	4.5	8.8	6.4	2.7	2.9
Cl (mg/L)	0.57	0.87	0.35	0.31	0.27	0.21	0.29	1.03	0.37	0.24
TDS (mg/L)	42	47	28	39	28	32	42	36	34	298
DOC (mg/L)	9.9	7.3	12.4	11.7	10.1	9.6	7.8	9.6	5.0	8.3
Color Pt-Co	48.6	12.7	86.4	58.4	46.5	42.9	25.9	53.9	9.7	30.9
PH	7.8	7.87	7.36	7.69	7.40	7.57	7.75	7.53	7.90	7.79
Secchi (m)	2.4	3.5	2.3	2.5	3.1	2.6	2.6	2.8	9.0	3.4

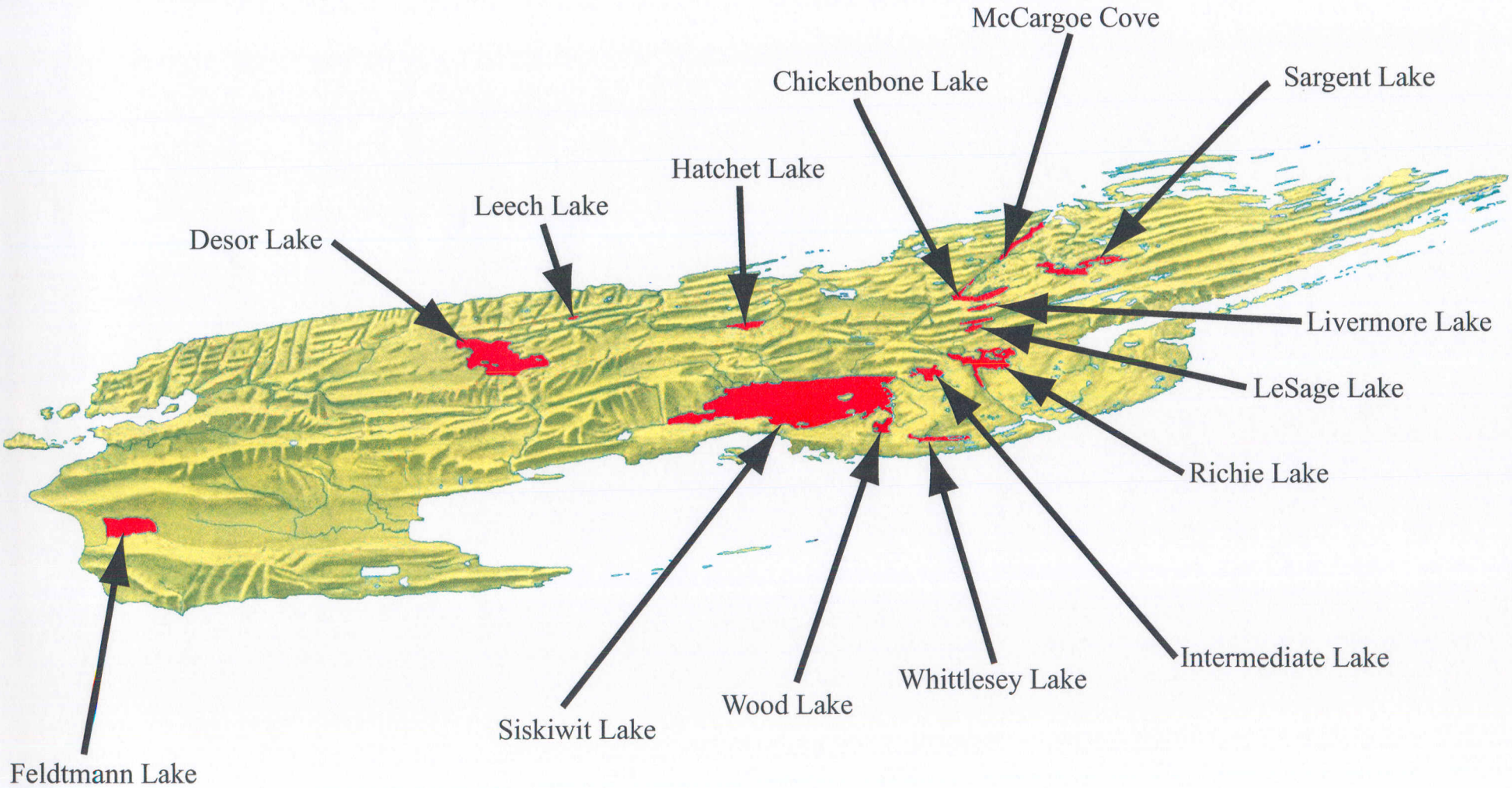


Figure 1. ISRO Sample sites.

METHODS

The sampling program in ISRO included initial visual scouting of rivers and lakes in order to determine where unionids are located, followed by intensive sampling by SCUBA divers in waters where unionids are found. This intensive sampling involved qualitative and quantitative components using stratified selection of sampling stations.

The location of quantitative samples, associated GPS coordinates, and further intensive diver surveys of unionid areas are presented in Figures 2-11. Polygon maps representing the surface area of each lake were constructed using "Arc/Info" (ESRI, Inc) Geographic Information Systems (GIS) software. Geospatially referenced, digital orthophotography images (US Geological Survey, Mid-Continent Mapping Center, 1992 and 1998) of each lake were obtained and used for digitizing of the shoreline, which provided whole lake areal calculations. GPS positions collected at each of the sample sites were then overlain on top of these surface area polygons. Based on interviews with the SCUBA divers, boundaries were then drawn on the surface polygon combining the sample point locations and the diver transects to determine the 'qualitative' survey areas. These boundaries were joined to the whole lake surface polygon to obtain the aerial calculations for the qualitative surveys and percent of the lake covered by these surveys.

Quantitative data on unionid distribution and abundance was obtained by sampling a series of triangular grids placed along a transect extending perpendicular from shore, across various depth strata. Site selection of sampling stations relied on both distance and habitat. One-half of the sampling stations in each lake were selected based on distance- spaced either 10-minute (for smaller lakes) or 20-minute canoe journey apart. The second half of stations were selected based on different habitats to ensure that all habitat types present in each lake, (gravel, sand, emergent vegetation, submerged vegetation, creek outlets, etc.) were sampled. The number of stratified samples collected in each lake varied in relation to lake morphometrics, habitat types, and unionid distribution. The numbers and locations of these samples are presented in Figures 2-11. At each sample location, once the point along the shore was selected, the first square meter grid was set. Five replicate square meter grids at each depth strata were sampled

along a transect line leading from this point encompassing all depth strata at 1.5 meter (5 ft.) intervals. Initial grid size was 25 m^2. which was dropped to 0.8 m^2 due to the large number of unionids obtained, and to more accurately define habitat. All changes noted in the data set. The number of grids sampled in each water body is presented in Table 4. Large numbers of clams (>300/grid) cannot be sampled fast enough to minimize stress to the animals. All unionids found were collected and species type, shell length, sex (if shell dimorphic) gravidity, and any other characteristics noted for every animal. Except for a few representative animals, all unionids were returned to the substrate.

Table 4. The total area sampled in each of the seven bodies of water sampled in Isle Royale National Park, USA. The size of the grids (m^2) used and the total number of the grids sampled by each body of water.

Lake	0.8-m2 grids	5-m^2 grids	25-m^2 grids	Total Area Sampled (m^2)
Lesage	0	0	16	400
Livermore	0	4	9	245
Chickenbone	0	34	0	170
McCargoe Cove	0	6	0	30
Richie	0	33	0	165
Intermediate	0	29	0	145
Whittlesey	0	19	0	95
Siskiwit	525	0	0	420
Desor	235	0	0	188

Population statistics included descriptive statistics (mean, median, quartile, range, etc.) as well as t-tests, moving averages, ANOVA, ANCOVA, Fischer's LSD, and other statistical methods to analyze the abundance data (number of mussels/taxon/transect), comparisons between populations within a water body and water bodies and potential relationships to habitats (see Appendix 2). Tracking unionid distribution by depth strata is not possible at this time since bathymetric data is not available for most of the lakes sampled.

Species identification was based on live shell and collected dead shell. Shell was brought to the mollusk experts at University of Michigan, Ann Arbor, MI, and University

of Ohio, Columbus Ohio, for verification of identification. Voucher specimens for each type of live shell found have been collected and will be submitted separately.

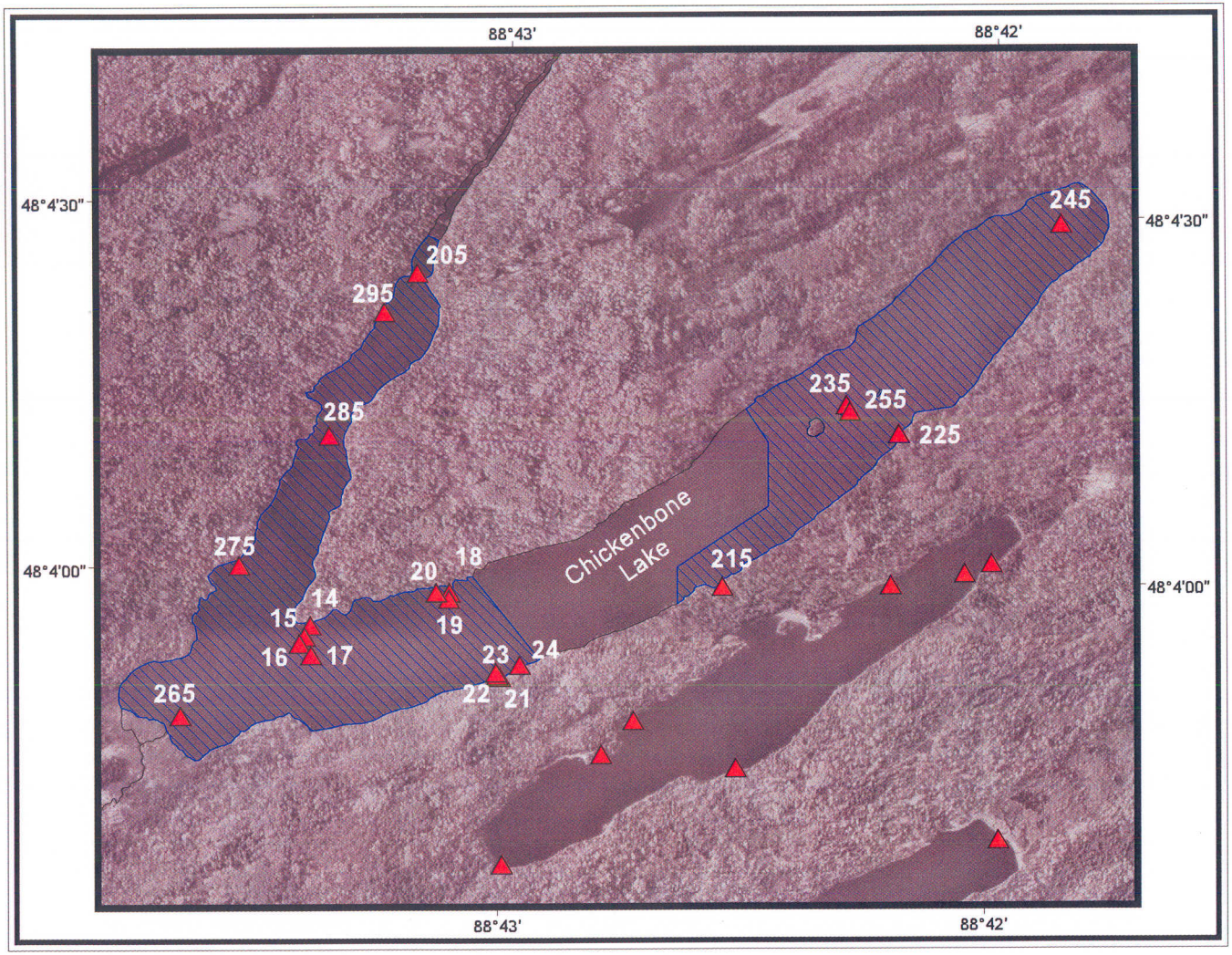
Estimates of age and growth rates for representative clams from each site were determined by sectioning the shell on a line from the umbo to the ventral margin of the shell. The cut sections were ground and polished using a series of fine grade emery papers, followed by polishing with a felt wheel and jewelers rouge. The shell sections were then examined under a 10-60 X power dissecting scope. Internal annular rings were determined using techniques described in Tevesz and Carter (1980). Length and age frequencies were then plotted using a curvilinear regression. Comparisons between internal and external annuli (examination for non-annular external rings) were done according to the techniques described in Downing et al. (1992).

The amount and type of chemical contaminant present per gm. of soft body tissue for each species sampled was determined for clams from each water body sampled. Individual clams were collected, placed on ice as quickly as possible and sent to the Great Lakes Science Center (GLSC). Soft tissues from each animal were removed from the shell and frozen at –40°F. The following contaminant array will be surveyed: pesticides including hexachlorobenzene, pentachlorobenzene, octachlorostryene, α- and γ-BHC, aldrin, dieldrin, endrin, α- and β-heptachlor epoxide, cis- and trans- nonachlors, p,p'- (DDE, DDD, and DDT), mirex (including 8-monohydro mirex), α- and γ-chlordanes, oxychlordane, toxaphenes (Cl 6 to Cl 10), dacthal, and pentachlorophenyl methyl ether; PCBs (80 congeners, including most of the planar dangerous ones) and mercury. Analysis techniques and QA/QC protocols are described in Schmidt (1997), Schmidt and Hesselberg (1992), and Wilford et al. (1973).

Tissue samples were also sent out to a contract laboratory (Edglo Laboratories, Fort Wayne, Indiana) for metal analysis that could not be done at the GLSC. Tissues were analyzed for barium, cadmium, chromium, copper, lead, mercury, nickel, and zinc using ICP coupled with ultrasonic nebulization sample introduction. This technique allows for ICP multi-parameter analysis with graphite absorption spectroscopy detection limits.

The contaminant concentrations in clam tissues were screened for toxicity by comparison with sediment benchmark values for toxicity to freshwater biota. These benchmark values had been assembled by scientists at the Great Lakes Science Center in two tables, used for screening residues in Lake Erie/Lake St. Clair NAWQA and the Illinois River basin NAWQA. The former table relied on benchmarks from the Oak Ridge National Laboratories (URL http://www.hsrd.ornl.gov/ecorisk/tm95r4.pdf) and NOAA (URL http//www.orca.nos.noaa.gov/projects/nsandt/sedimentquality.html). The latter table incorporated consensus-based sediment quality guidelines for freshwater ecosystems from the following reference: MacDonald DD, Ingersoll CG, Berger TA (2000) Arch. Environ. Contam. Toxicol. 39:20-31. For chemicals without consensus values, then available values from the first table were used. In evaluating the residues in the lakes, we did not normalize by organic content of the sediment, although some benchmark values are normalized to 1% organic carbon.

Figure 2. Sample Sites for Chickenbone Lake (2000), Isle Royale National Park.



0 0.25 0.5 0.75 1 Kilometers

N W E S

Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

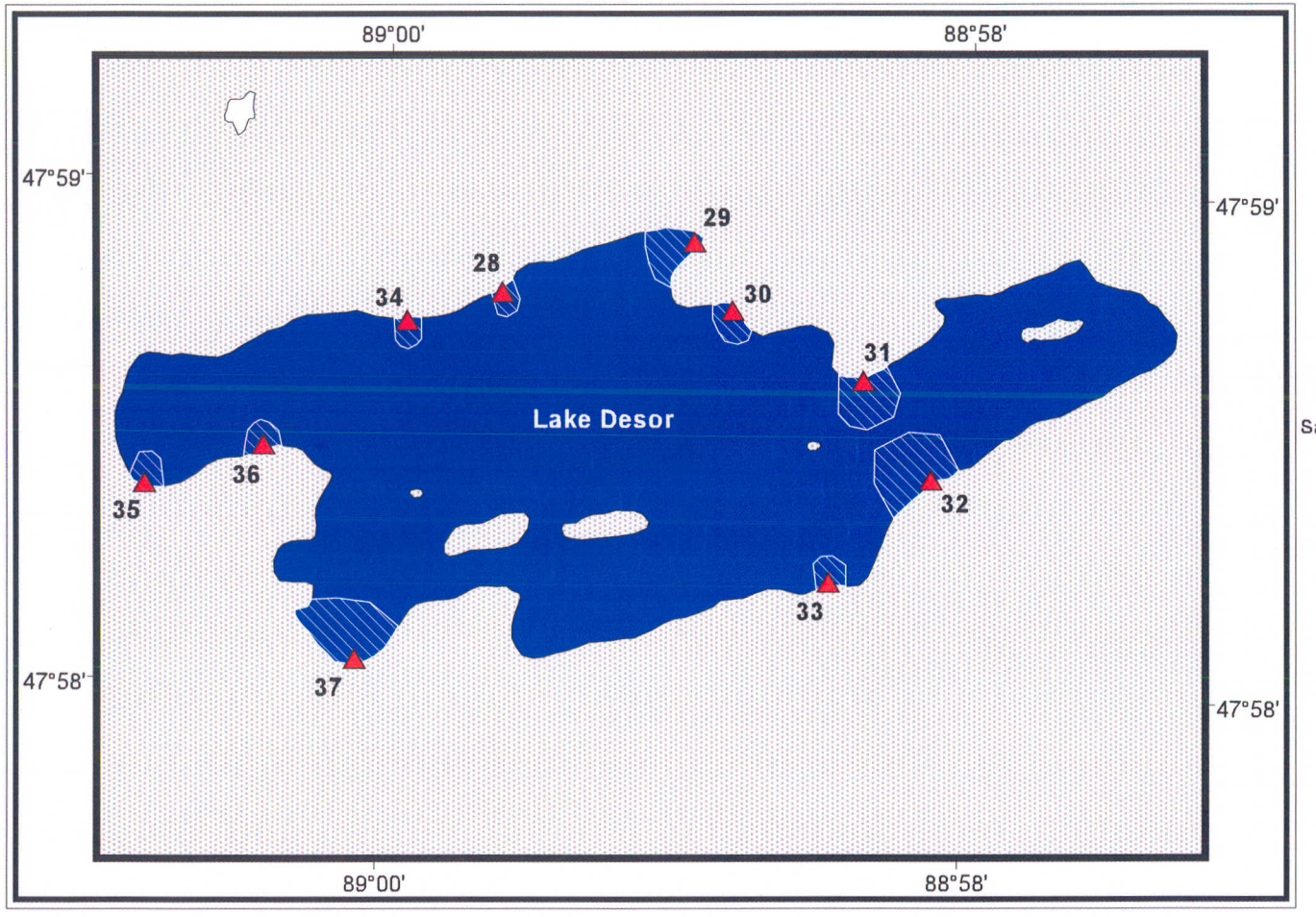
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15.	48° 3' 57.71"	88° 43' 24.44"
16.	48° 3' 57.08"	88° 43' 25.08"
17.	48° 3' 56.15"	88° 43' 23.58"
18.	48° 4' 1.55"	88° 43' 6.54"
19.	48° 4' 0.99"	88° 43' 6.64"
20.	48° 4' 1.52"	88° 43' 8.20"
21.	48° 3' 54.68"	88° 43' 0.18"
22.	48° 3' 54.80"	88° 43' 0.51"
23.	48° 3' 55.01"	88° 43' 0.67"
24.	48° 3' 55.69"	88° 42' 57.76"
215.	48° 4' 2.44"	88° 42' 32.90"
225.	48° 4' 15.27"	88° 42' 11.49"
235.	48° 4' 17.52"	88° 42' 17.99"
245.	48° 4' 32.71"	88° 41' 51.85"
255.	48° 4' 17.02"	88° 42 17.52"
265.	48° 4' 50.98"	88° 43' 39.59"
275.	48° 4' 3.43"	88° 43' 32.70"
285.	48° 4' 14.22"	88° 43' 21.86"
295.	48° 4' 24.42"	88° 43' 15.23"
205.	48° 4' 27.64"	88° 43' 11.24"

Digital orthophotography data source:
US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM (zone 16)
North American Datum, 1983

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Figure 3. Sample Sites for Lake Desor (2001), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

Sample ID#:	Latitude (N):	Longitude (W):
28.	47° 58' 51.54"	88° 59' 36.30"
29.	47° 58' 57.55"	88° 58' 56.44"
30.	47° 58' 49.04"	88° 58' 48.56"
31.	47° 58' 41.63"	88° 58' 22.69"
32.	47° 58' 27.35"	88° 58' 07.10"
33.	47° 58' 11.48"	88° 58' 27.91"
34.	47° 58' 47.19"	88° 59' 55.64"
35.	47° 58' 22.96"	89° 00' 49.07"
36.	47° 58' 29.25"	89° 00' 24.61"
37.	47° 57' 58.88"	89° 00' 05.15"

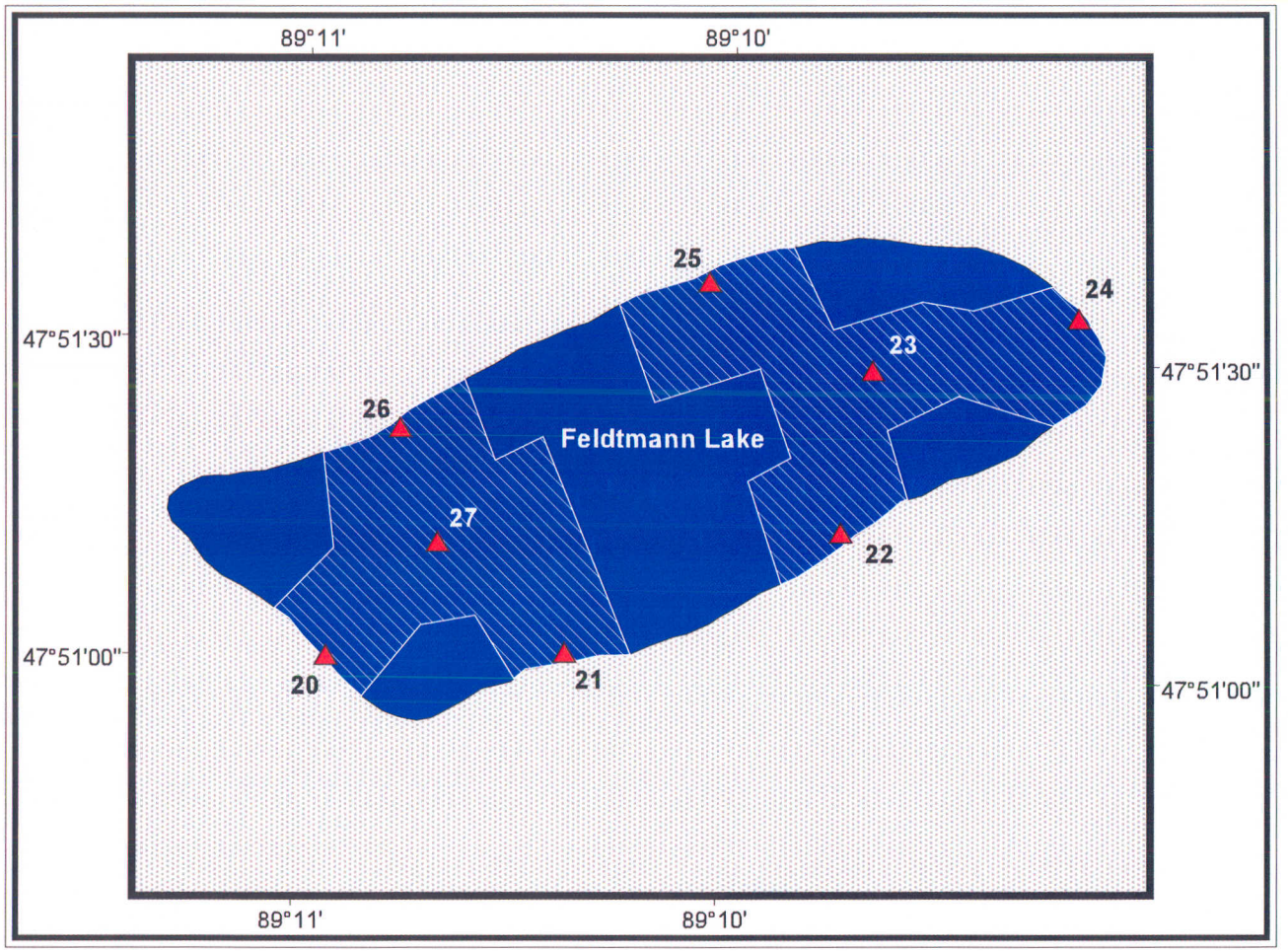
0 0.5 1 1.5 2 Kilometers

Digital orthophotography data source: US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM (zone 16)
North American Datum, 1983

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Figure 4. Sample sites for Feldtmann Lake (2001), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

Sample ID#:	Latitude (N):	Longitude (W):
20.	47° 50' 54.27"	89° 10' 56.12"
21.	47° 50' 59.78"	89° 10' 23.45"
22.	47° 51' 14.35"	89° 09' 45.59"
23.	47° 51' 26.25"	89° 09' 34.20"
24.	47° 51' 32.63"	89° 09' 13.05"
25.	47° 51' 35.60"	89° 10' 04.33"
26.	47° 51' 23.87"	89° 10' 44.56"
27.	47° 51' 13.67"	89° 10' 40.51"

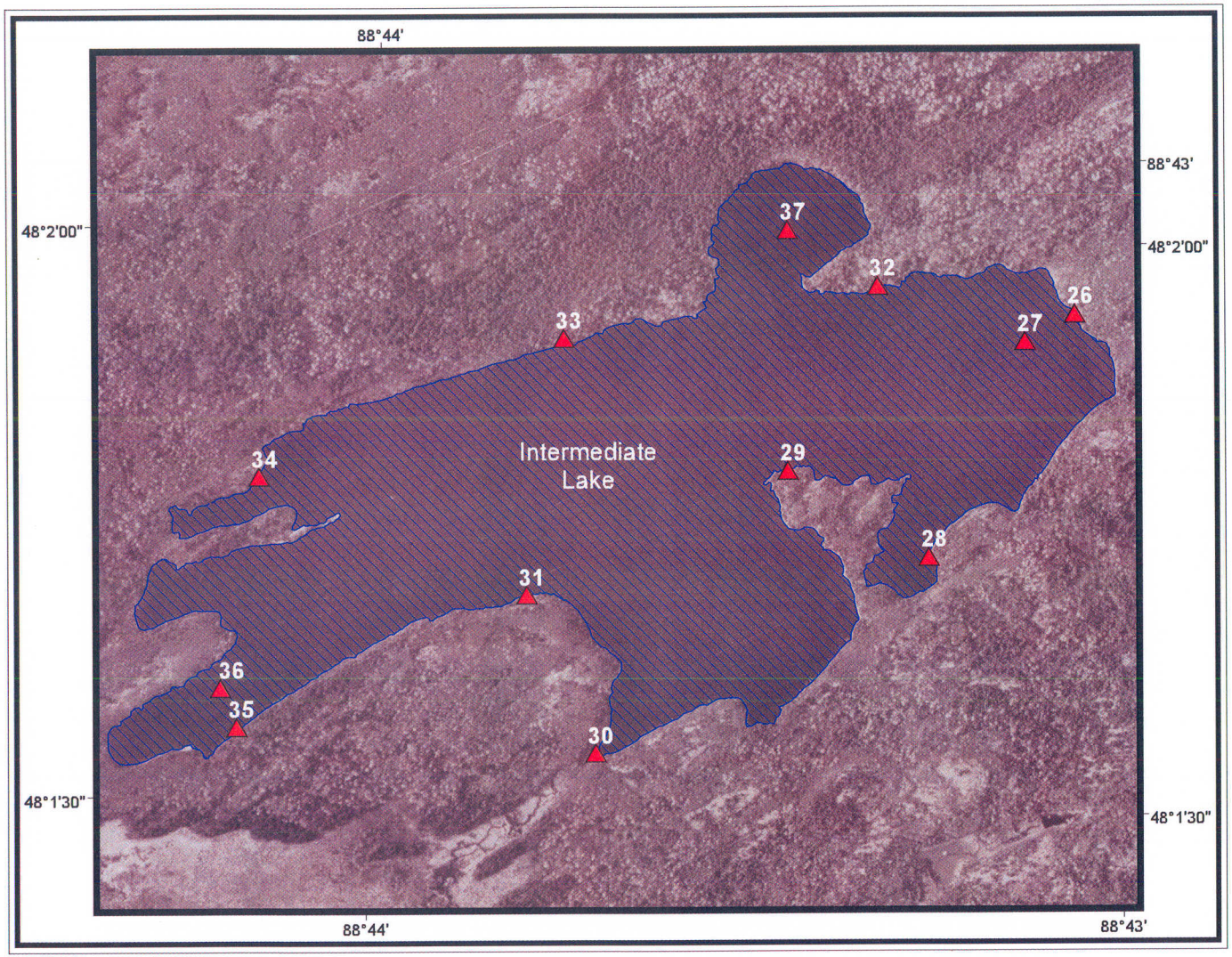
0 0.25 0.5 0.75 1 Kilometers

Digital orthophotography data source: US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM (zone 16)
North American Datum, 1983

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Figure 5. Sample sites for Intermediate Lake (2000), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

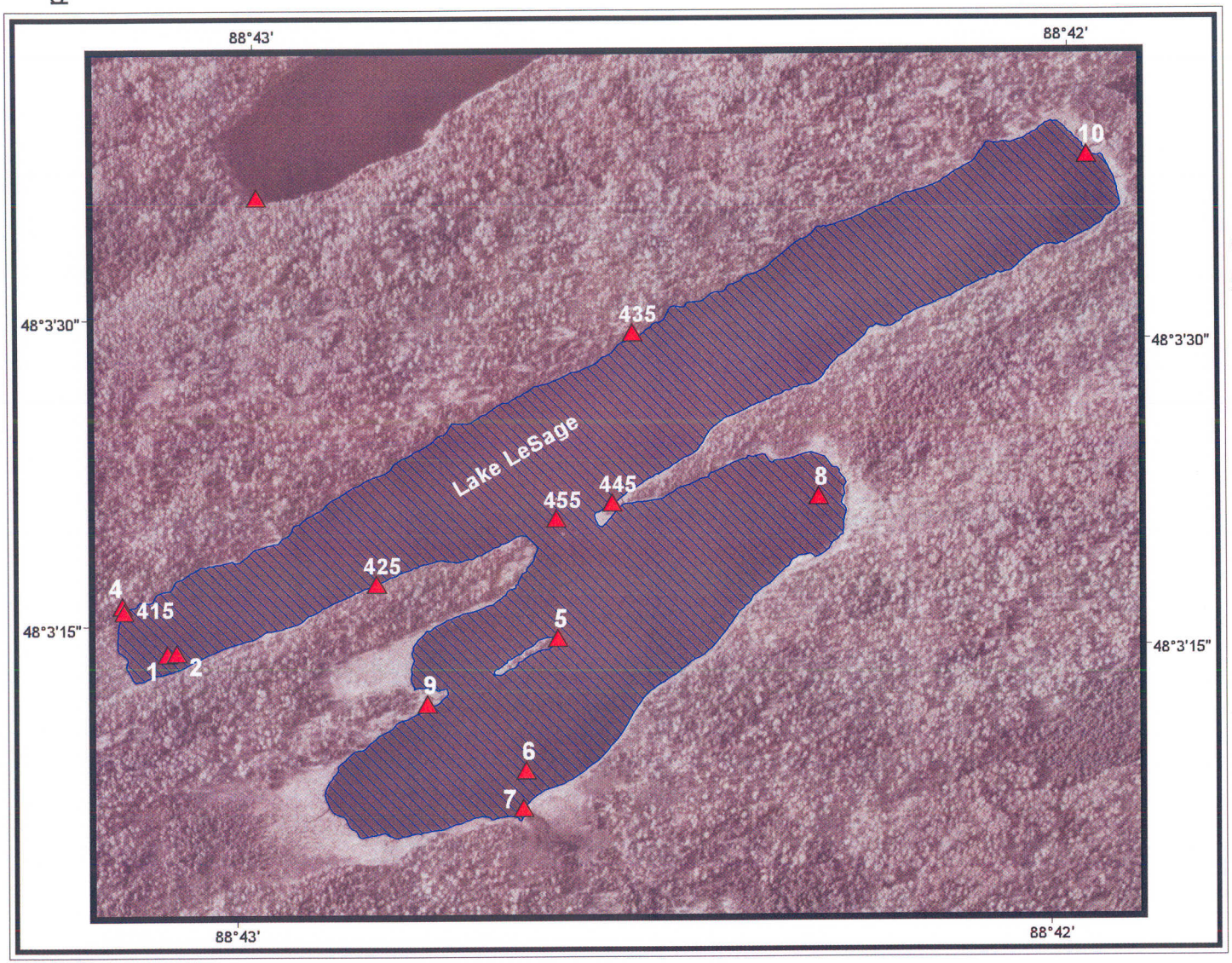
Sample ID#:	Latitude (N):	Longitude (W):
26.	48° 1' 59.47"	88° 43' 4.74"
27.	48° 1' 57.95"	88° 43' 8.62"
28.	48° 1' 46.52"	88° 43' 15.90"
29.	48° 1' 50.91"	88° 43' 27.19"
30.	48° 1' 35.85"	88° 43' 41.99"
31.	48° 1' 44.08"	88° 43' 47.71"
32.	48° 2' 0.72"	88° 43' 20.36"
33.	48° 1' 57.62"	88° 43' 45.12"
34.	48° 1' 50.04"	88° 44' 9.03"
35.	48° 1' 36.82"	88° 44' 10.41"
36.	48° 1' 38.88"	88° 44' 11.79"
37.	48° 2' 3.56"	88° 43' 27.55"

Digital orthophotography data source:
US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM
North American Datum, 1983

USGS science for a changing world

Figure 6. Sample Sites for Lake LeSage (2000), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

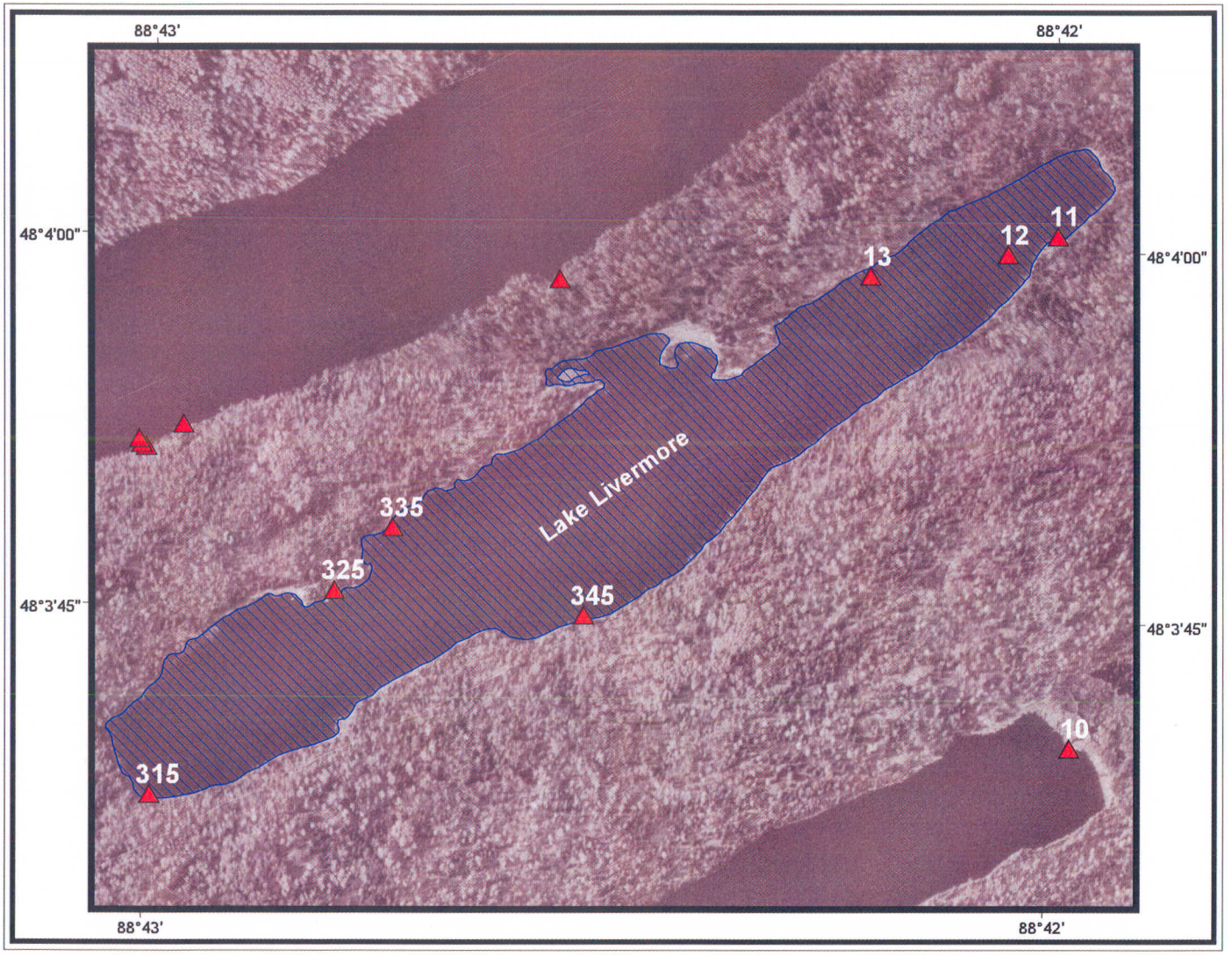
Sample ID#:	Latitude (N):	Longitude (W):
1.	48° 3' 16.85"	88° 43' 5.46"
2.	48° 3' 16.91"	88° 43' 4.78"
4.	48° 3' 19.19"	88° 43' 8.88"
5.	48° 3' 18.03"	88° 42' 36.71"
6.	48° 3' 11.54"	88° 42' 38.88"
7.	48° 3' 9.69"	88° 42' 39.04"
8.	48° 3' 25.24"	88° 42' 17.67"
9.	48° 3' 14.68"	88° 42' 46.28"
10.	48° 3' 42.21"	88° 41' 58.36"
415.	48° 3' 18.88"	88° 43' 8.77"
425.	48° 3' 20.49"	88° 42' 50.13"
435.	48° 3' 33.07"	88° 42' 31.59"
445.	48° 3' 24.71"	88° 42' 32.86"
455.	48° 3' 23.88"	88° 42' 36.98"

Digital orthophotography data source:
US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM
North American Datum, 1983

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Figure 7. Sample Sites for Lake Livermore (2000), Isle Royale National Park



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

Sample ID#:	Latitude (N):	Longitude (W):
11.	48° 4' 4.81"	88° 41' 59.79"
12.	48° 4' 3.96"	88° 42' 3.10"
13.	48° 4' 2.88"	88° 42' 12.19"
315.	48° 3' 39.31"	88° 42' 59.50"
325.	48° 3' 48.49"	88° 42' 47.48"
335.	48° 3' 51.34"	88° 42' 43.67"
345.	48° 3' 47.63"	88° 42' 30.87"

Digital orthophotography data source: US Geological Survey, Mid-Continent Mapping Center, 1992

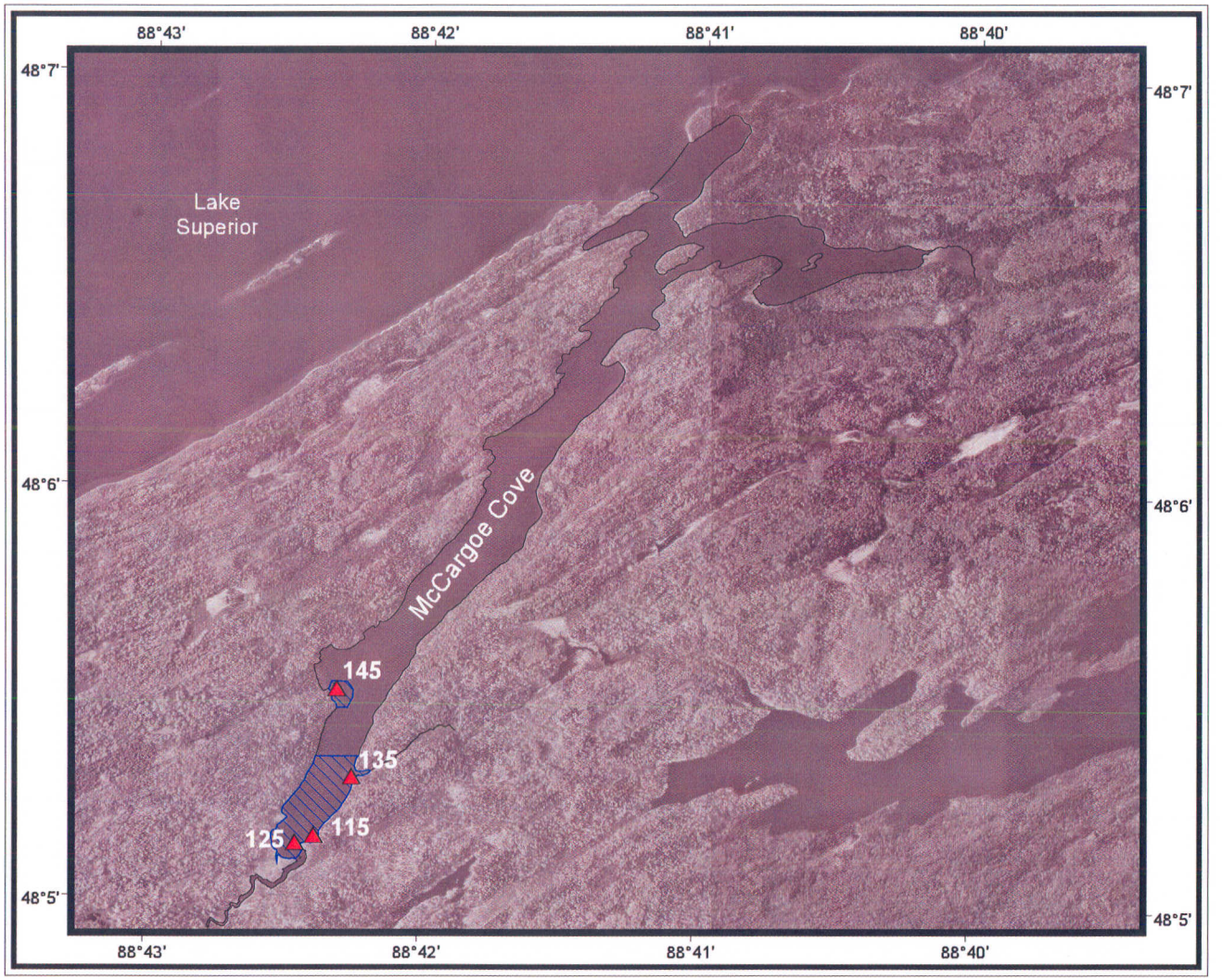
Projection: UTM
North American Datum, 1983

0 0.25 0.5 0.75 1 Kilometers

N W E S

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Figure 8. Sample Sites for McCargoe Cove (2000), Isle Royale National Park.



0 0.5 1 1.5 2 Kilometers

N
W E
S

Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

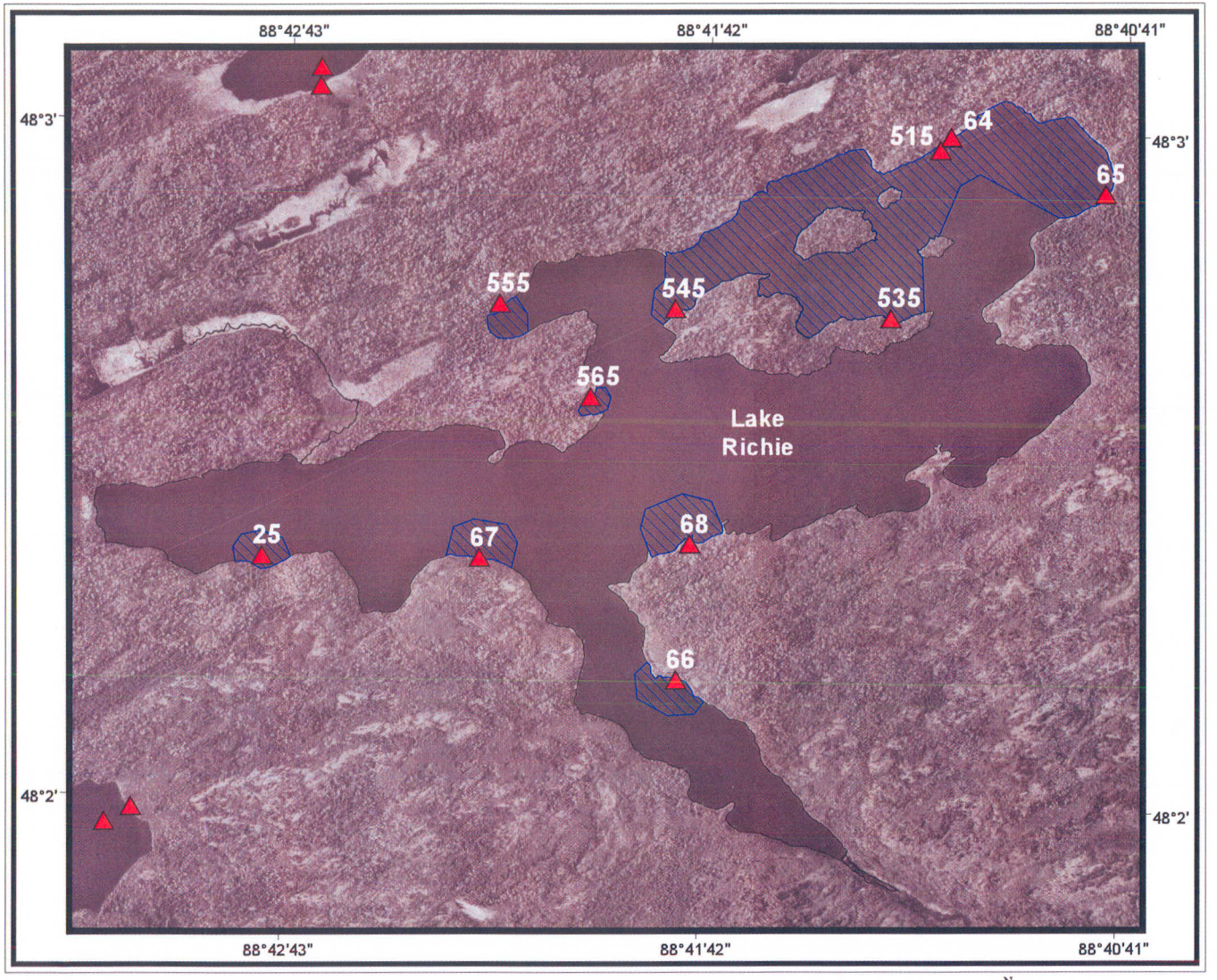
Sample ID#:	Latitude (N):	Longitude (W):
115.	48° 5' 12.55"	88° 42' 23.13"
125.	48° 5' 11.47"	88° 42' 27.15"
135.	48° 5' 21.17"	88° 42' 15.09"
145.	48° 5' 33.89"	88° 42' 18.72"

Digital orthophotography data source: US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM
North American Datum, 1983

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Figure 9. Sample Sites for Lake Richie (2000), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

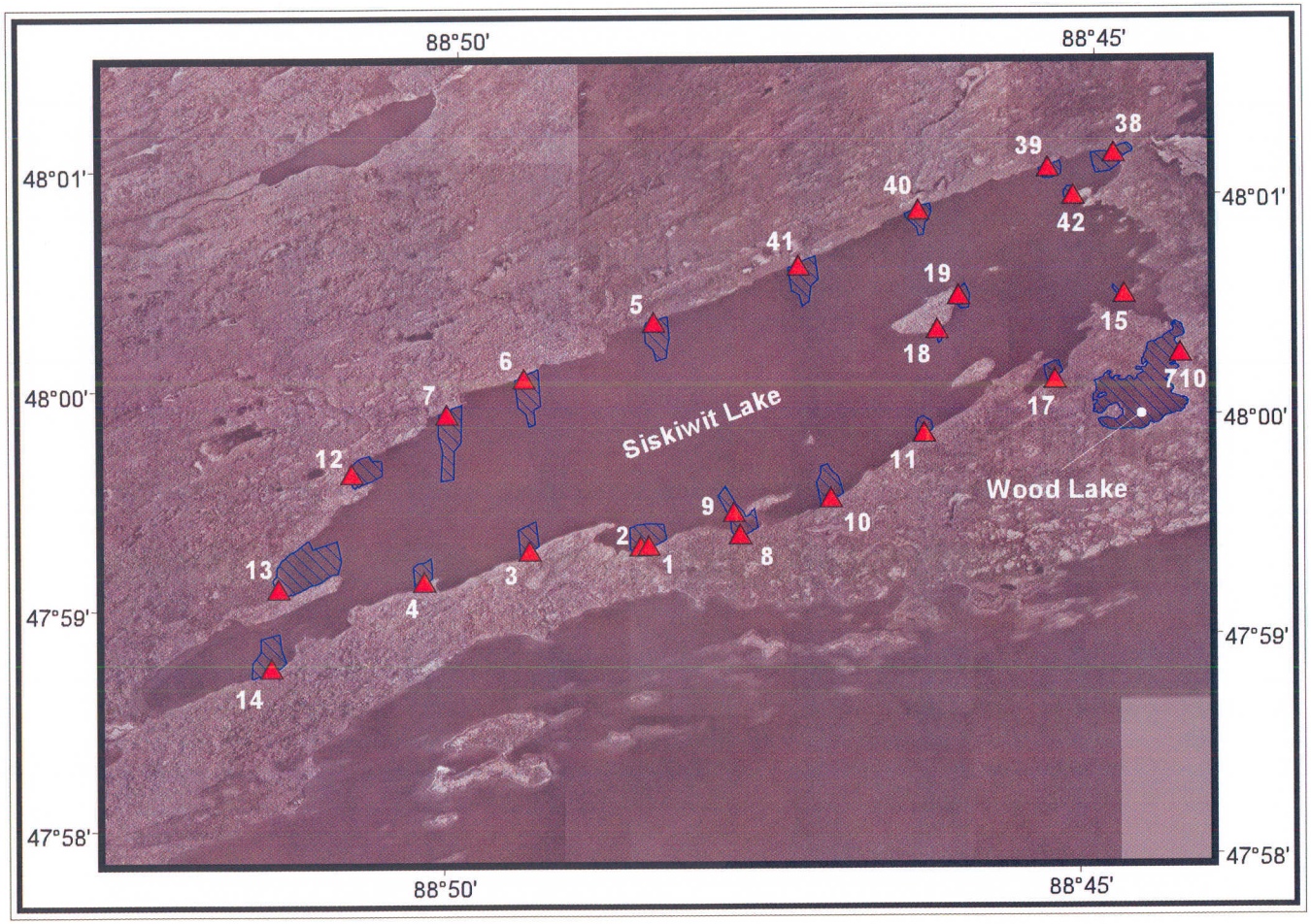
Sample ID#:	Latitude (N):	Longitude (W):
25.	48° 2' 24.08"	88° 42' 46.48"
64.	48° 3' 5.90"	88° 41' 6.96"
65.	48° 3' 0.68"	88° 40' 44.25"
66.	48° 2' 12.79"	88° 41' 45.62"
67.	48° 2' 24.29"	88° 42' 14.64"
68.	48° 2' 26.00"	88° 41' 44.00"
515.	48° 3' 4.65"	88° 41' 8.51"
535.	48° 2' 48.30"	88° 41' 15.25"
545.	48° 2' 48.79"	88° 41' 46.71"
555.	48° 2' 48.95"	88° 42' 12.31"
565.	48° 2' 40.08"	88° 41' 58.88"

Digital orthophotography data source:
US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM
North American Datum, 1983

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Figure 10. Sample Sites for Wood Lake (2000) and Siskwet Lake (2001), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

Sample ID#:	Latitude (N):	Longitude (W):
Siskiwit Lake:		
01.	47° 59' 19.26"	88° 48' 26.64"
02.	47° 59' 19.36"	88° 48' 28.99"
03.	47° 59' 16.86"	88° 49' 22.40"
04.	47° 59' 06.25"	88° 50' 11.71"
05.	48° 00' 30.68"	88° 48' 27.28"
06.	48° 00' 12.36"	88° 49' 27.28"
07.	48° 00' 00.59"	88° 50' 02.91"
08.	47° 59' 23.73"	88° 47' 43.81"
09.	47° 59' 30.15"	88° 47' 46.20"
10.	47° 59' 35.70"	88° 47' 00.66"
11.	47° 59' 56.73"	88° 46' 17.18"
12.	47° 59' 40.26"	88° 50' 48.57"
13.	47° 59' 03.59"	88° 51' 20.43"
14.	47° 58' 38.84"	88° 51' 22.44"
15.	48° 00' 41.69"	88° 44' 45.29"
17.	48° 00' 15.37"	88° 45' 16.09"
18.	48° 00' 30.24"	88° 46' 13.16"
19.	48° 00' 40.38"	88° 46' 02.83"
38.	48° 01' 26.81"	88° 44' 52.68"
39.	48° 01' 21.59"	88° 45' 21.76"
40.	48° 01' 07.60"	88° 46' 22.66"
41.	48° 00' 49.45"	88° 47' 18.43"
42.	48° 01' 12.28"	88° 45' 10.30"
Wood Lake:		
710.	48° 00' 23.37"	88° 44' 17.25"

0 1 2 3 4 5 Kilometers

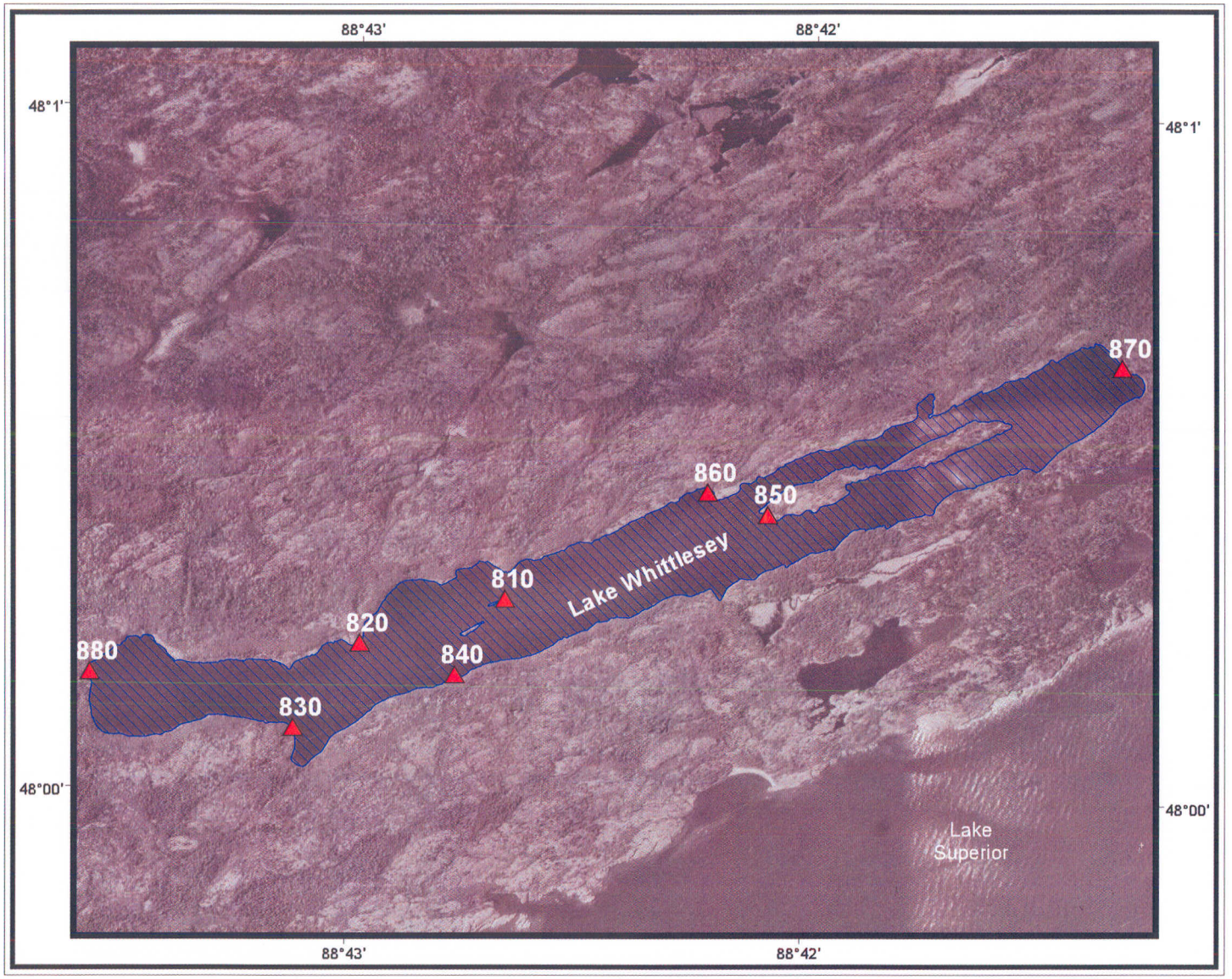
N W E S

Digital orthophotography data source:
US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM (zone 16)
North American Datum, 1983

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Figure 11. Sample Sites for Lake Whittlesey (2000), Isle Royale National Park.



Legend

- Mussel sample location (Quantative)
- Qualitative survey area (USGS - SCUBA)

Sample Location Information:

Sample ID#:	Latitude (N):	Longitude (W):
810.	48° 0' 20.37"	88° 42' 39.82"
820.	48° 0' 16.23"	88° 42' 58.82"
830.	48° 0' 8.68"	88° 43' 7.40"
840.	48° 0' 13.62"	88° 42' 46.24"
850.	48° 0' 28.25"	88° 42' 5.32"
860.	48° 0' 30.11"	88° 42' 13.29"
870.	48° 0' 41.77"	88° 41' 18.87"
880.	48° 0' 13.23"	88° 43' 34.38"

Digital orthophotography data source:
US Geological Survey, Mid-Continent Mapping Center, 1992

Projection: UTM
North American Datum, 1983

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RESULTS

Unionids were found in nine of the twelve sites, being absent from Feldtmann, Hatchet, and "Leech" lakes. In addition, during the initial scout trip during 1999, various outlet streams associated with these lakes were qualitatively surveyed, and no unionids were seen in these streams. No exotic bivalves, zebra mussels or Asian clams specifically, were found in any inland lake or adjacent Lake Superior waters.

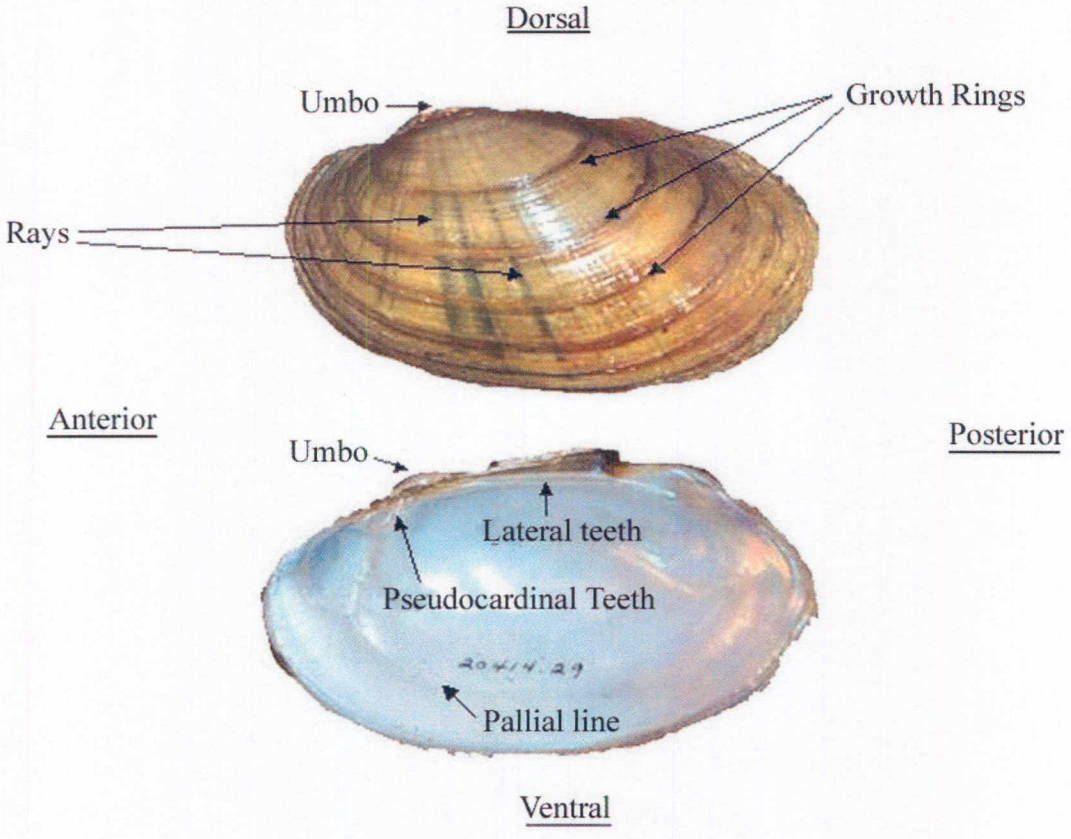
Taxonomic Identifications

A figure showing the major morphological features of unionid is included to aid in deciphering descriptions (Figure 12). To date, two unionid genera have been identified in Isle Royale waters, *Lampsilis* (muckets) and *Pyganodon* (floaters), with four species designated. None of these genera or species are considered endangered, threatened, or species-of-concern either at the state or federal level. Species identifications and pictures are provided in Figures 13-17, with locality distributions presented in Table 5. In general, the high inland lakes contained only *Pyganodon cataracta*; other species of *Pyganodon*, plus the *Lampsilis* species were found in lakes lower in elevation, and closer to Lake Superior.

Table 5. The distribution of unionid species found in Isle Royale National Park, 2000-2001. X= present. O= absent.

Lake	*L. luteola*	*L. radiata*	*P. cataracta*	*P. grandis*	*P.* Intergrades
Chickenbone Lake	X	X	X	X	X
Desor Lake	O	O	X	O	O
Feldtmann Lake	O	O	O	O	O
Hatchet Lake	O	O	O	O	O
Intermediate Lake	X	X	X	X	X
"Leech" Lake	O	O	O	O	O
Livermore Lake	O	O	X	O	O
LeSage Lake	O	O	X	O	O
McCargoe Cove	X	X	X	X	X
Richie Lake	O	X	X	X	X
Siskiwit Lake	X	X	X	X	X
Lake Desor	O	O	X	O	O
Whittlesey Lake	O	O	X	X	X

Figure 12. Unionid Shell Morphology.



Taxonomic Authority

The genera designations are not in taxonomic dispute, and are easily identified in the field (Figures 13-17). However, the species identifications for the *Lampsilis* group and the *Pyganodon* group are in major taxonomic dispute, and have undergone many name changes during the last 50 years, with more changes likely in the future, until this issue is resolved. Furthermore, the two main taxonomic authorities for the Midwest, the Ohio State University Museum of Biological Diversity, The Bivalve Mollusk Collection (OSU), Drs. Stansbury and Watters, and the University of Michigan Mollusk Collection (UM) Dr. Burch, R. Sherman, D. Graf, do not agree on species designations for these genera. Since we expect the names to shift in the next decade, and because it is easier to combine data, than to try to split past collections and reports, we have chosen to use the OSU nomenclature (splitters rather than clumpers). Thus, for this report, we are using the following names:

1. ***Lampsilis***

There are two visually distinct forms of *Lampsilis* at ISRO that can be readily identified in the field (Figures 13-15). For this reason, we used the OSU system, and have divided the *Lampsilis* into two species, *L. radiata* and *L. luteola.* At the University of Michigan Mollusk Collection (Dan Graf and Renée Sherman) all these animals would be lumped together and called *Lampsilis siliquoidea*. Some taxonomists list *L. radiata* and *L. luteola* as sub-species of *L. siliquoidea*.

The shell of *L. luteola* is light colored (tan), usually with visible greenish stripes, and the shell hinge tooth, the pseudocardinal tooth, is broad with strong secondary crenellations (Figures 13-15). The shell of the second species, *L. radiata* is usually dark colored (dark red brown) and with faint if any stripes. Overall, the shell is more angular in appearance (front to back) and has a thick, heavy robust hinge line. The pseudocardinal tooth is narrower, more triangular, with minimal secondary crenellations.

Lampsilis luteola and *L. radiata* distribution patterns were identical, found in the same lakes, same grids, coexisting side-by-side, with no differences related to sex or age. No obvious hybrid forms were found. *Lampsilis* are one of the few genera of unionids whose shells are sexually dimorphic. Figure 15 shows how to sex these clams externally.

2. ***Pyganodon*** **(previously called** ***Anodonta*****)**

Identification of *Pyganodon* species is difficult not only due to poor taxonomic revision, extensive shell variability within the different species, natural erosion of key shell characteristics, and the observation that hybridization between species is a common occurrence. We have identified two species, *P. grandis*, and *P. cataracta*, plus *P. grandis/cataracta* intergrades or hybrids (Figure 16). These species and hybrids are not as readily differentiated in the field, as are the *Lampsilis* species. Identifications are based on shell shape, which is often subjective, and the whorls located on the umbo of the shell (beak structure), which are often eroded even in very young animals (Figure 17). The hybrids are so designated because they combine the shell shape and/or beak structure of both species.

Figure 13. *Lampsilis spp.* collected at Isle Royale National Park, 2000.

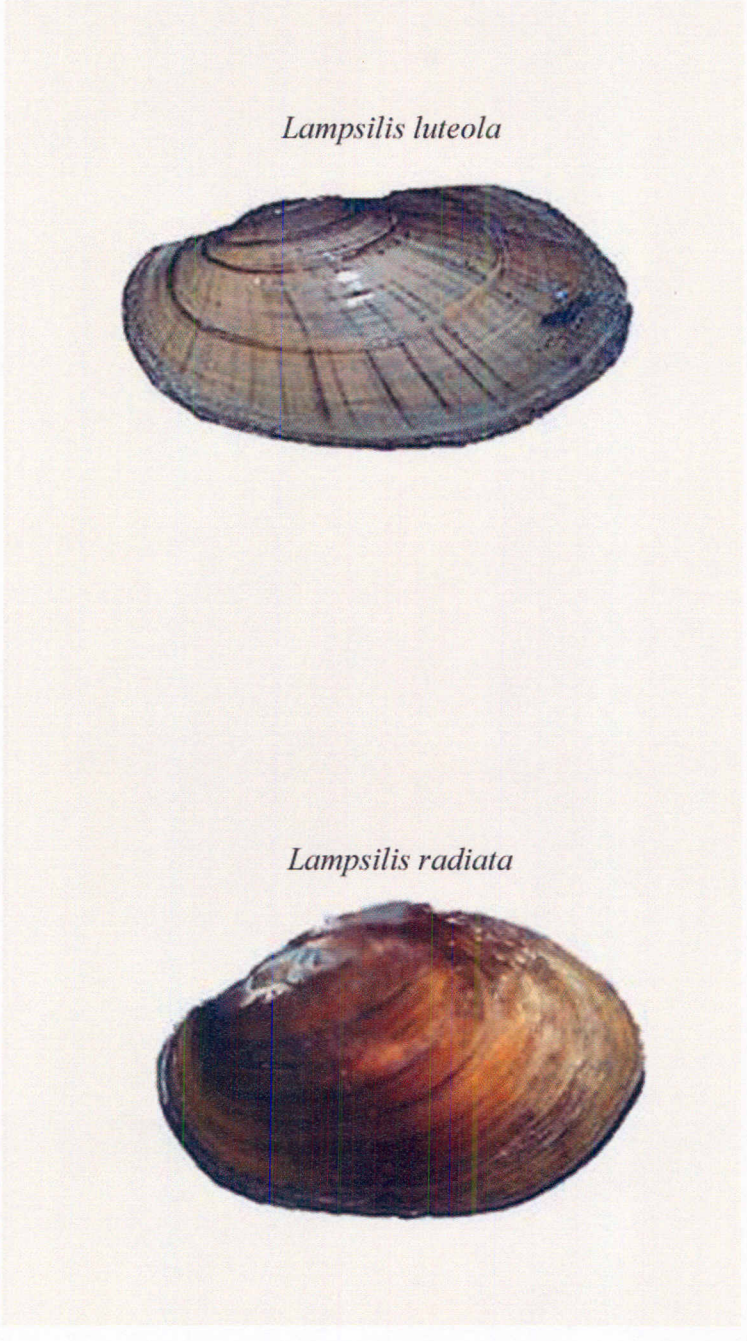


Figure 14. Shell tooth types found in *Lampsilis luteola* and *L. radiata*.

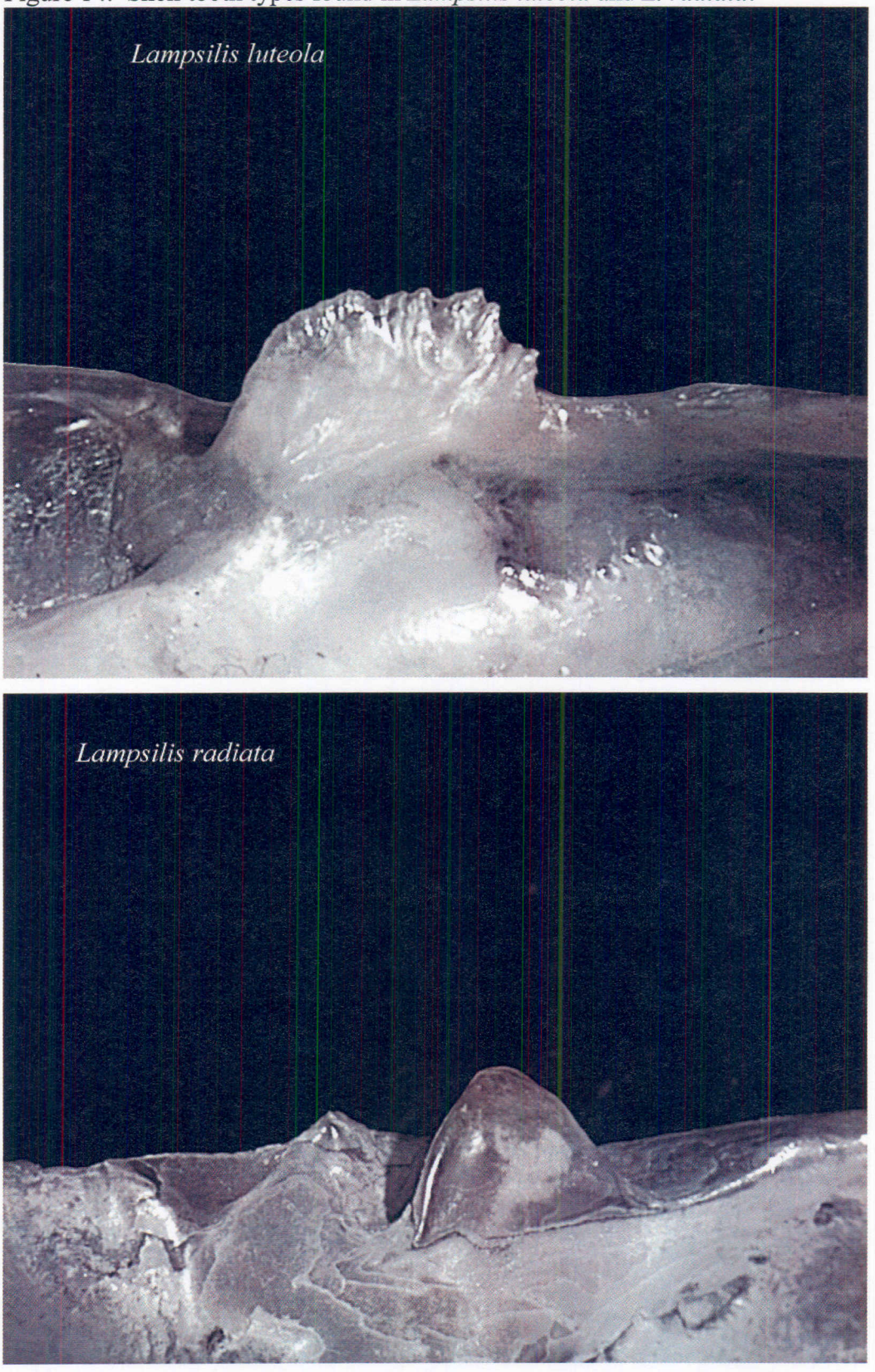
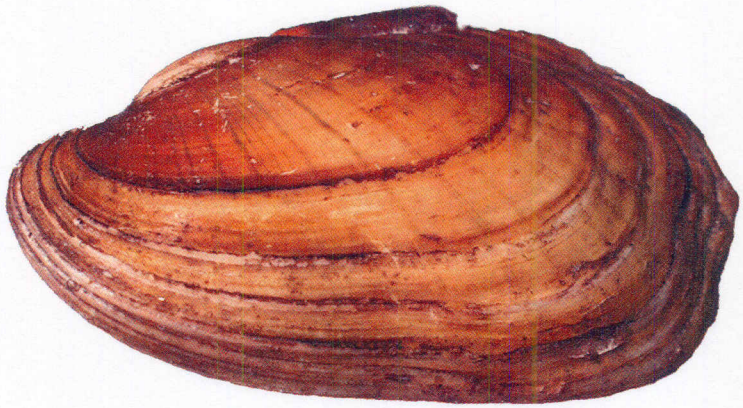


Figure 15. Sexual dimorphism in *Lampsilis spp.* shells.

Female



Male



Figure 16. *Pyganodon spp.* collected at Isle Royale National Park, 2000.

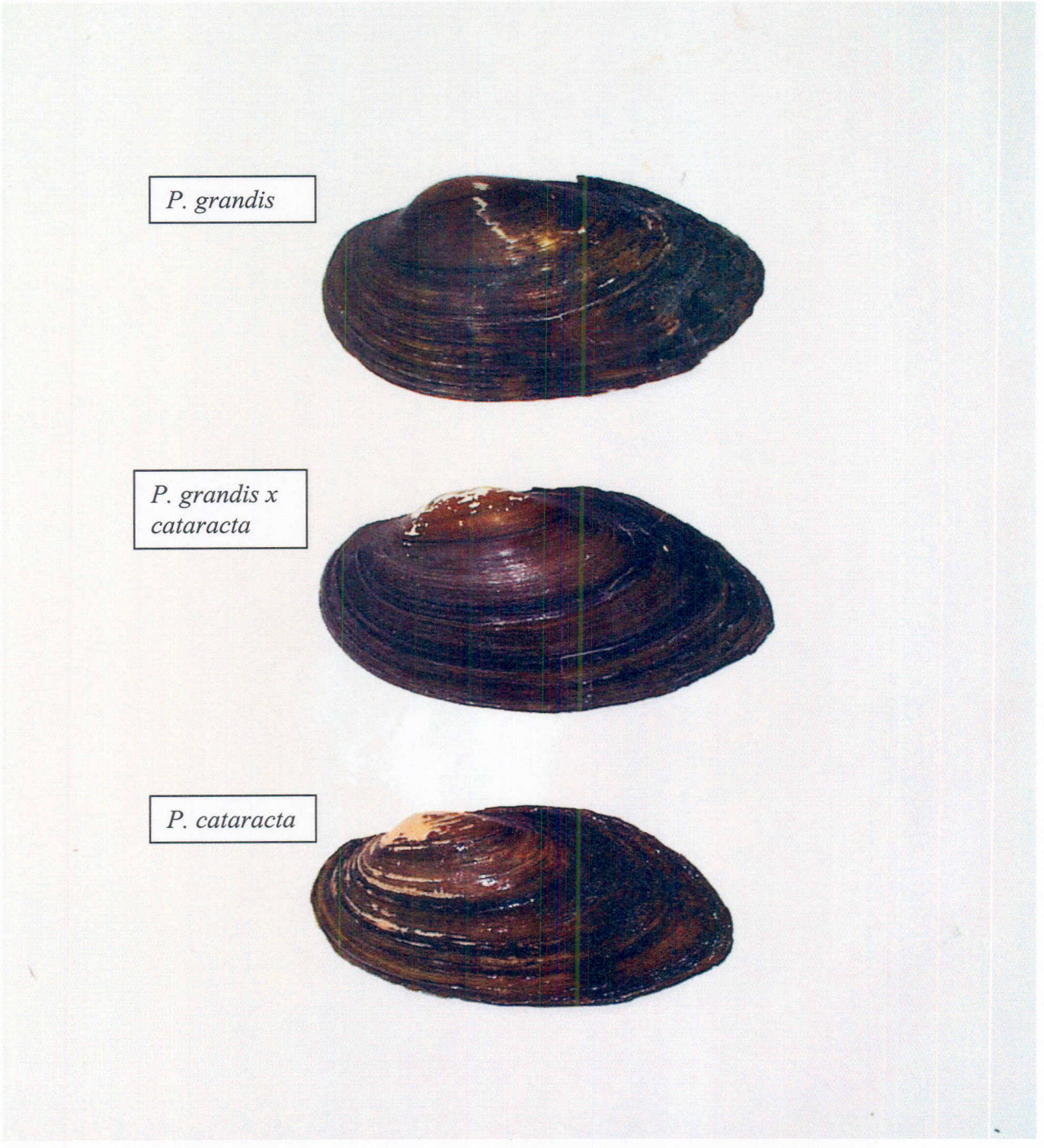
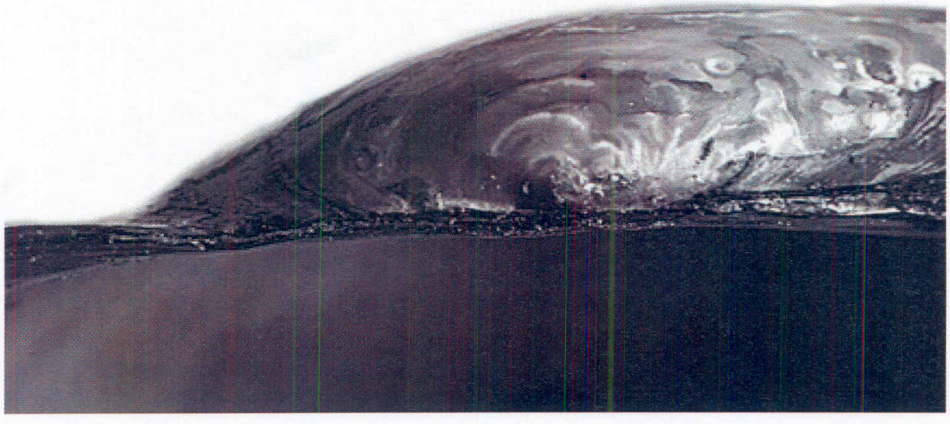
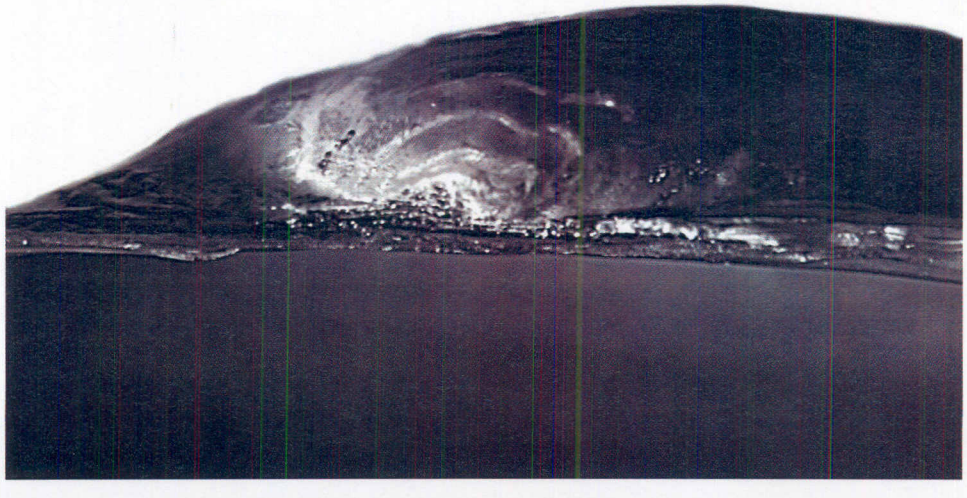


Figure 17. Beak structure in *Pyganodon spp.* collected in Isle Royale National Park, 2000.

Pyganodon grandis



Pyganodon cataracta



Habitat Preference

The unionids were not randomly distributed within the lakes or in McCargoe Cove. Depth, and by inference thermocline, appears to be the main feature controlling distribution within each lake in ISRO. Most unionids were located above the thermocline in deeper lakes, with no depth limitations in shallower lakes where thermoclines were not detected. No thermocline development was detected in Chickenbone, LeSage, Livermore, Intermediate, Richie, and Whittlesey lakes. With the exception of Whittlesey, clams of both genera (where present) were found across all depth profiles, with no statistically noticeable difference ($p<0.05$) in depth distribution. The distribution of clams in Lake Whittelsey is a special case, affected more by lake area and substrate type rather than depth, and is discussed below.

No thermocline was detected in Desor, though one would expect it to occur due to its deep depth (Table 2). However, wind and waves action was high during the sampling period and may have caused enough mixing to mask thermocline development. Clam distribution was significantly limited by depth, with clams not found below 9.1 m.

Well-developed thermoclines were detected in Siskiwit and McCargoe Cove. Lake Siskiwit actually had several layers of sharp drops in water temperature, the first at 8.5m, the second at 10.3m, and the third at 13.7m. Clams did not occur below 9.1m. In McCargoe Cove, the thermocline was at about 9.0m and clams were not found in deeper waters.

Substrate type did affect distribution of the genus *Lampsilis spp.*, but not *Pyganodon spp.* The presence of *Lampsilis spp.* was positively correlated with rocky/cobble areas, particularly in Lake Chickenbone, where *Lampsilis spp.* are only found in the center of the lake, in the rocky areas along the 0-1.5m. depth strata (Table 6). *Lampsilis spp.* also dominated the clam population of Lake Siskiwit, which has substantial rocky substrate. It is the only water body sampled where *Lampsilis spp.* occurred in greater numbers than *Pyganodon spp.* The distribution of *Pyganodon spp.* above the thermocline appears to be random and could not statistically be correlated to substrate type.

Table 6. Total number of *Lampsilis* spp. found at 1.5m depth strata in bodies of water of Isle Royale National Park, USA. Common letters indicate no statistical difference ($p \leq 0.05$) in mean clam population density between water bodies.

WATER BODY	0 – 1.5 m	1.5 – 3m	3.0 – 4.5m	TOTAL
Chickenbone Lake	213 [c]	43 [b]	7 [a]	263
McCargoe Cove	0 [a]	1 [a]	0 [a]	1
Intermediate Lake	7 [a]	25 [b]	1 [a]	33
Total	220	68	8	296

Lake Whittlesey is somewhat different than the other water bodies sampled in that clam distribution was significantly correlated to lake region and substrate rather than depth. The clam population is concentrated in one area in the western end of the lake, though a few scattered individuals can be found elsewhere. This lake did not appear to stratify, and has both rocky and sandy areas. However, the center section of this lake is steep sided, with sheets of shear bedrock on the northern side and a broken rock substrate that is very unstable and subject to avalanches at the slightest touch on the southern side. It is not the fact that the substrate is rock that is the problem; it is the instability. The eastern end of this lake consists of a shallow sand bar that appears to be wind-scoured. The western end of the lake where most of the clams are found is a deeper bay, with stable sand/gravel substrate.

Population Densities

Population densities varied by lake and by genus. Lake Chickenbone had the highest densities/m^2; Lake Whittlesey the lowest (Table 7; Figure 18) (Desor and Siskiwit data has not yet been analyzed). Maximum #/m^2 for all lakes sampled was found along the rocky shoreline in 0-10 ft of water Lake Chickenbone. The potential relationships between environmental parameters such as chlorophyll *a* concentrations as presented in Tables 2 and 3 and clam #/m^2 were examined using statistical correlation and cluster analyses, but no significant relationship was detected.

Table 7. Kolmogorov-Smirnov Two Sample Test comparison of native clam populations (#/m^2) of nine water bodies associated with Isle Royal National Park. Common letters indicate no statistical difference ($p \leq 0.05$) in mean clam population density between water bodies.

	LeSage	Livermore	Chickenbone	McCargoe	Richie	Intermediate	Whittlesey	Siskiwit	Desor
Total Clams Sampled	680	1597	1412	31	293	767	77	928	200
Minimum/m^2	0.52	0.79	1.60	0.00	0.00	0.00	0.00	0.00	0.00
Maximum/m^2	4.70	5.47	33.20	2.40	1.25	4.73	0.93	31.25	33.75
Median/m^2	1.28	2.18	5.80	0.50	0.40	0.87	0.10	1.25	0.00
Mean/m^2	1.66	2.44	8.59	0.82	0.43	1.13	0.26	1.63	1.06
Standard Error	0.31	0.34	1.38	0.342	0.05	0.19	0.07	0.01	0.19
Standard Deviation	1.17	1.21	8.06	0.838	0.27	1.04	0.30	2.51	2.97
	b,c			a,b,d	d	b	e	c,d,e	a

Density/m^2 is not necessarily a good predictor of overall population size. The total amount of acceptable habitat has a greater influence than just #/m^2. Estimating total population size can only be done in those lakes where no significant differences in distribution by depth contour or substrate could be detected. Overall population estimates can only be estimated for those lakes with random distribution, not related to thermocline or substrate, which basically limits population calculations to Intermediate, LeSage, and Livermore lakes, and if limited only to *Pyganodon spp.*, to Lake Chickenbone. Population size for depth stratified lakes requires detailed bathymetric data which is not yet available for other lakes. Based on GPS aerial lake size estimates as provided in Table 2, in combination with the mean #/m2 from Table 7, total population estimates are provided in Table 8. The average population estimate for Lake Chickenbone of 6,388,200 is low since *Lampsilis spp.* numbers are not included. The distribution of this clam genus is limited to rocky areas and without detailed substrate maps, we cannot estimate population numbers. Much of Chickenbone is soft sediments, especially the western arm, and contain few if any *Lampsilis spp.*

Table 8. Estimated population size of unionids found in several lakes in Isle Royale National Park, USA. The population estimate for Lake Chickenbone only includes *Pyganodon spp.* densities, and does not include *Lampsilis spp.*

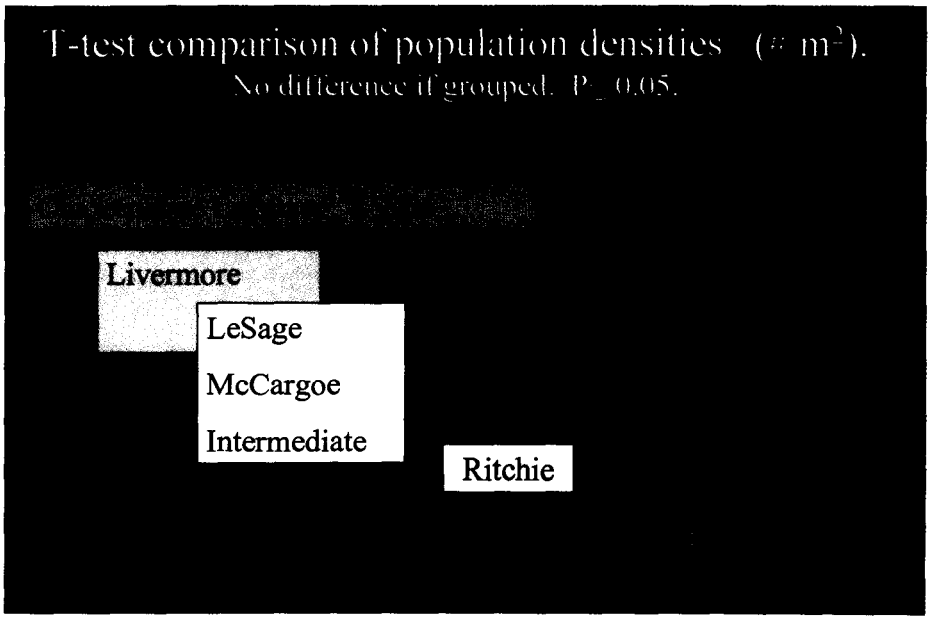
WATER BODY	POPULATION SIZE
Chickenbone Lake	6,388,200
Intermediate Lake	802,300
LeSage Lake	747,000
Livermore Lake	732,000

Population estimates are merely estimates, not hard numbers. Table 9 shows the variability and 95% confidence limits associated with our Chickenbone Lake estimates.

Table 9. Estimated population size of *Pyganodon spp.* in Chickenbone Lake, Isle Royale National Park, USA. This population estimate for Lake Chickenbone does not include *Lampsilis spp.* Number of cases=34. Ha=92.6

	# m	ESTIMATED POPULATION SIZE
Minimum	1.6	1,481,600
Maximum	25.6	23,705,600
Range	24.0	
Mean	7.02	6,388,200
95% Confidence Limits Upper	9.054	8,384,004
95% Confidence Limits Lower	4.99	4, 620,740
Standard Deviation	5.83	

Figure 18. T-test comparison of native clam populations (#/m^2) of seven water bodies associated with Isle Royal National Park. Overlapping groups indicate no statistical difference ($p \leq 0.05$) in mean clam population densities.



Age/Year Class

Unionid populations in all lakes surveyed to date showed signs of consistent recruitment and long-term survival. A number of gravid female unionids of both genera were seen during sampling, indicating conditions are suitable for reproductive efforts to be initiated. Actual time of release of gametes or glochidia could not be determined except in Lake Siskiwit, where *Lampsilis spp.* were seen releasing glochidia and found in multi-sex clusters (indication of spawning activity) in the last two weeks of July 2001. Figures 19-21 show length frequencies based on maximum shell length for species in different lakes indicating successful recruitment has been occurring almost annually and adults have survived for many decades. There was no significant difference in length frequency between species, lakes, or depths ($p<0.05$).

Figure 19. Length/frequency of *Pyganodon spp.* collected from Livermore Lake In Isle Royale National Park, 2000. Based on maximum shell length.

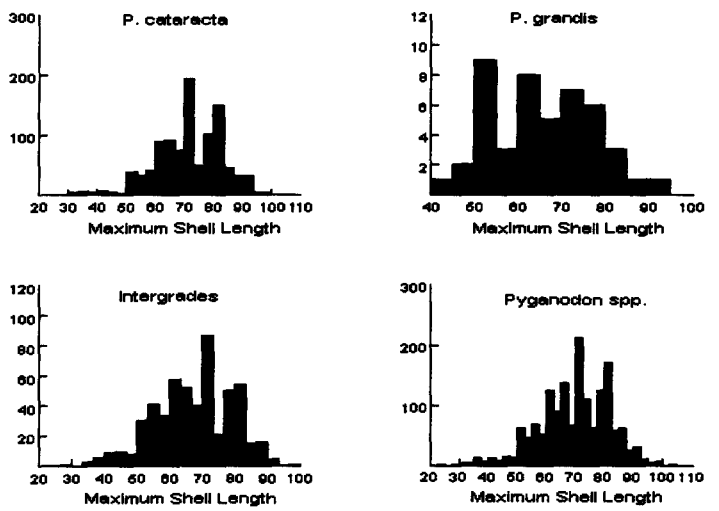
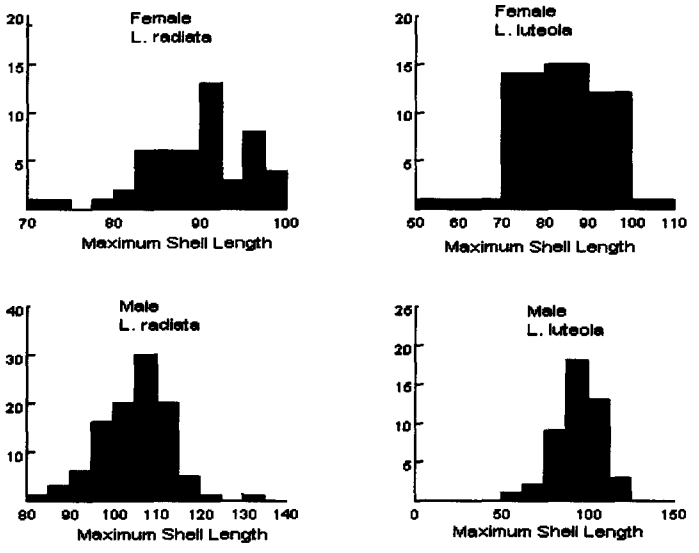


Figure 20. Length/frequency of *Lampsilis luteola* and *L. radiata* males and females collected from Chickenbone Lake In Isle Royale National Park, 2000. Based on maximum shell length.



Length and age are not necessarily directly related in unionids. To determine age, we first had to determine the relationship between external annuli or visible growth rings on the outside of the shell and a more accurate estimation of age as based on internal annuli in the shell cross-section. Using external annuli, if comparable, would give us a larger data set, since use of internal annuli requires the killing of the animal. Shell cross-sections showed that internal and external annuli were identical in *Pyganodon spp.*, but not in *Lampsilis spp,* except in lakes Siskiwit and Desor. Therefore, with *Pyganodon spp* and *Lampsilis spp.* from the lakes mentioned above, field notes on external annuli could be used to measure growth rates and age. Internal and external annuli for *Lampsilis spp* in the other lakes are in agreement up to about age 5 in *Lampsilis spp* (Figure 22). After this age, which is probably the age of sexual maturity, external annuli underrepresented age (internal annuli). Up until sexual maturity, unionids of both genera grew or deposited an average of about 10 mm in length of new shell annually.

Figure 21. Length/frequency of *Pyganodon cataracta* collected from Chickenbone Lake In Isle Royale National Park, 2000. Based on maximum shell length.

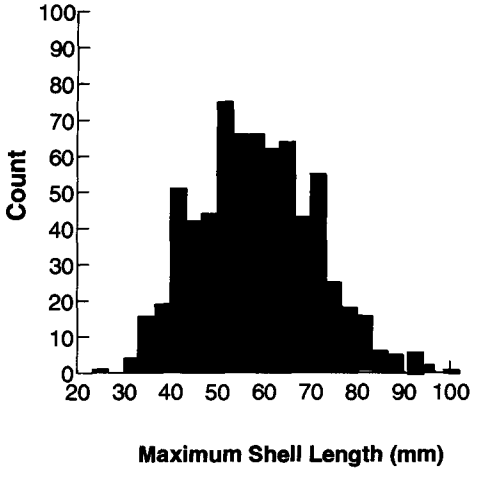
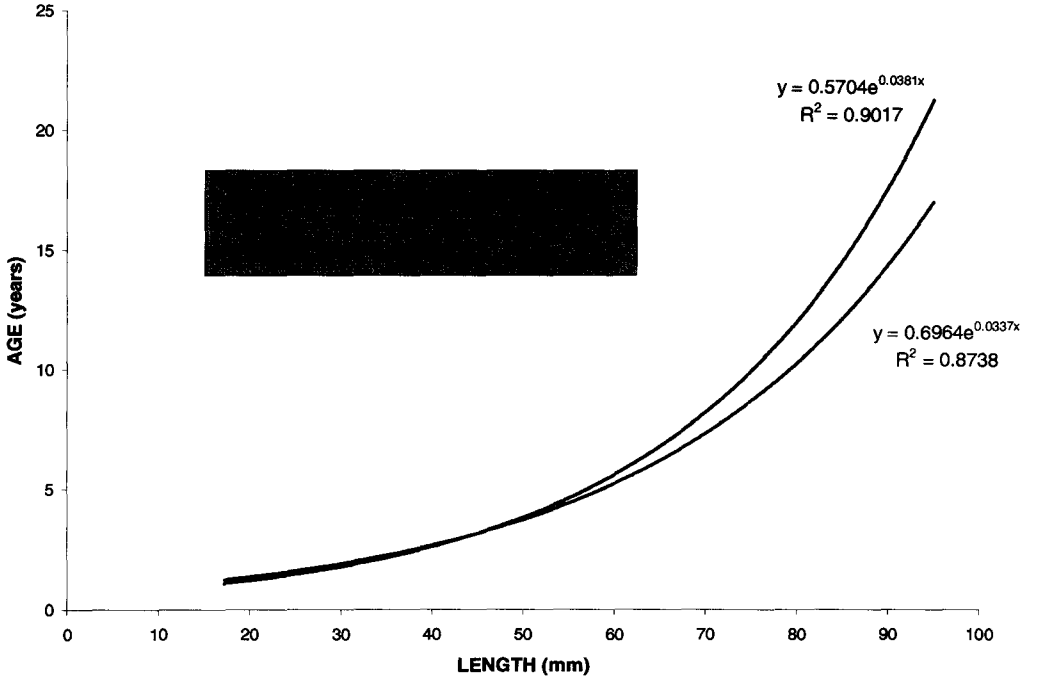


Figure 22. Comparison of the different age estimates obtained using either external or internal annuli from *Lampsilis spp.* in Chickenbone lake, 2000. Internal annuli are considered more accurate age indicators



The regression formulas presented in Figures 23-and 24 can be used to calculate an estimated age for unionids that were not killed and shelled-sectioned, so long as specific species and lake formulas are used. Based on these linear regressions, all of the *Pyganodon spp.* measured during our study were under 20 years of age, and the oldest *Lampsilis spp.* were about 62 years old. *Pyganodon spp.*, being a fast-growing, thin-shelled species, are known to be relatively short lived (Table 10).

Table 10. Oldest estimated age of unionids in various water bodies in Isle Royale National Park, 2000-2001, based on largest animal measured and length/age regressions determined by sectioning the shell of a subset of unionids from each lake.

WATER BODY	*Pyganodon spp.*		*Lampsilis spp.*	
Chickenbone Lake	118 mm	14.7 yrs	131 mm	43.7 yrs
Intermediate Lake	118 mm	13.9 yrs	117 mm	62.7 yrs
LeSage Lake	97 mm	13.2 yrs		
Livermore Lake	103 mm	12.8 yrs		
McCargoe Cove			101 mm	44.6 yrs
Richie Lake	106 mm	11.3 yrs		
Whittlesey Lake	87 mm	14.2 yrs		
Siskiwit Lake	90 mm	11.0 yrs	105 mm	15 yrs
Lake Desor	106 mm	16 yrs		

Figure 23. Age/length comparison for *Pyganodon spp.* between six lakes in Isle Royale National Park, 2000. Growth rates for Chickenbone (chkn) are significantly higher than for Intermediate, Lesage, Livermore (liver), Richie (Richie), or Whittlesey (wsee).

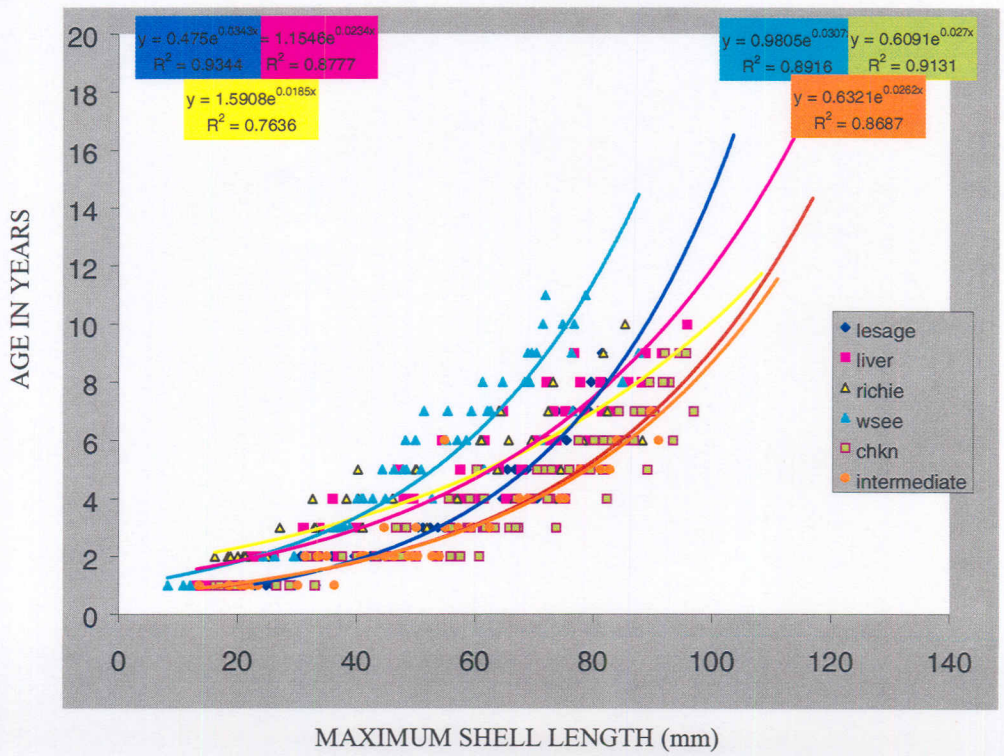
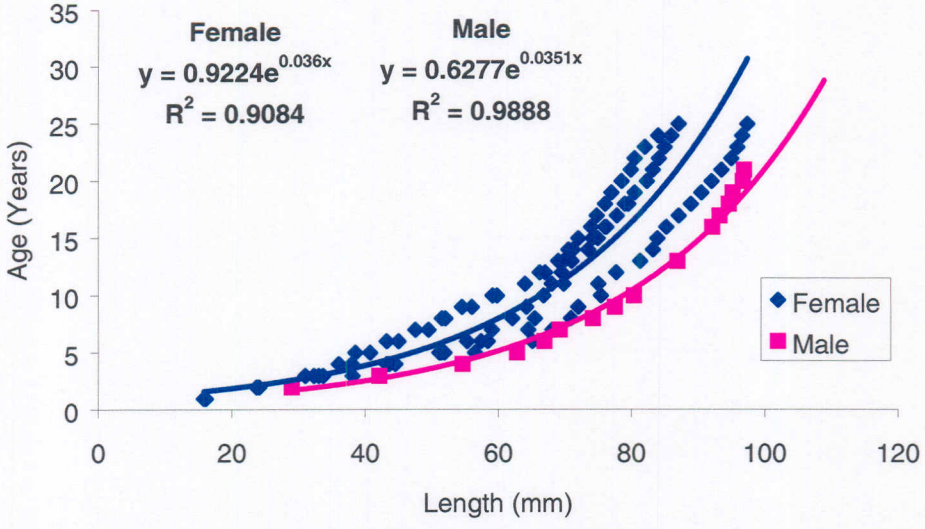


Figure 24. Age/length comparison for (A) *Lampsilis spp.*females and males, and (B) *Pyganodon spp.* between Siskiwit Lake and Desor Lake in Isle Royale National Park, 2001.

(A) *Lampsilis spp.* Age/Length comparison for Siskiwit Lake



(B) *Pyganodon spp.* Age/Length comparison for Desor Lake

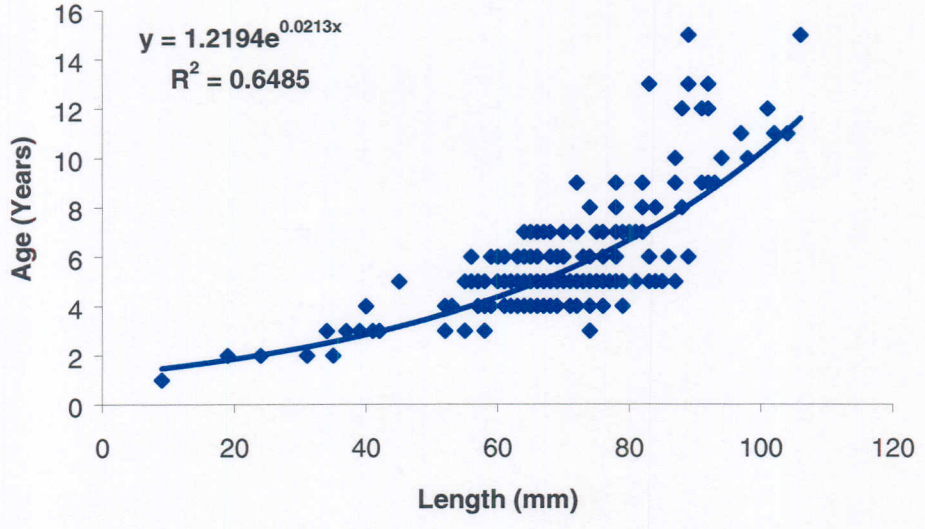
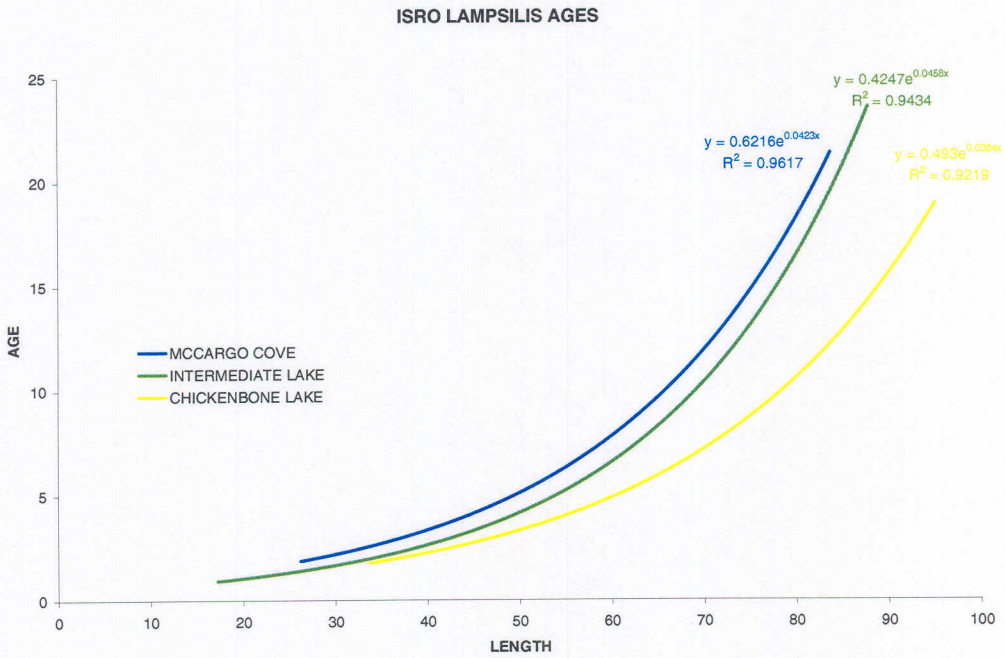


Figure 25. Age/length comparison for *Lampsilis spp*. between three water bodies in Isle Royale National Park, 2000. Chickenbone and Intermediate lakes are inland and McCargoe Cove is on Lake Superior.



Restrictions and Caveats

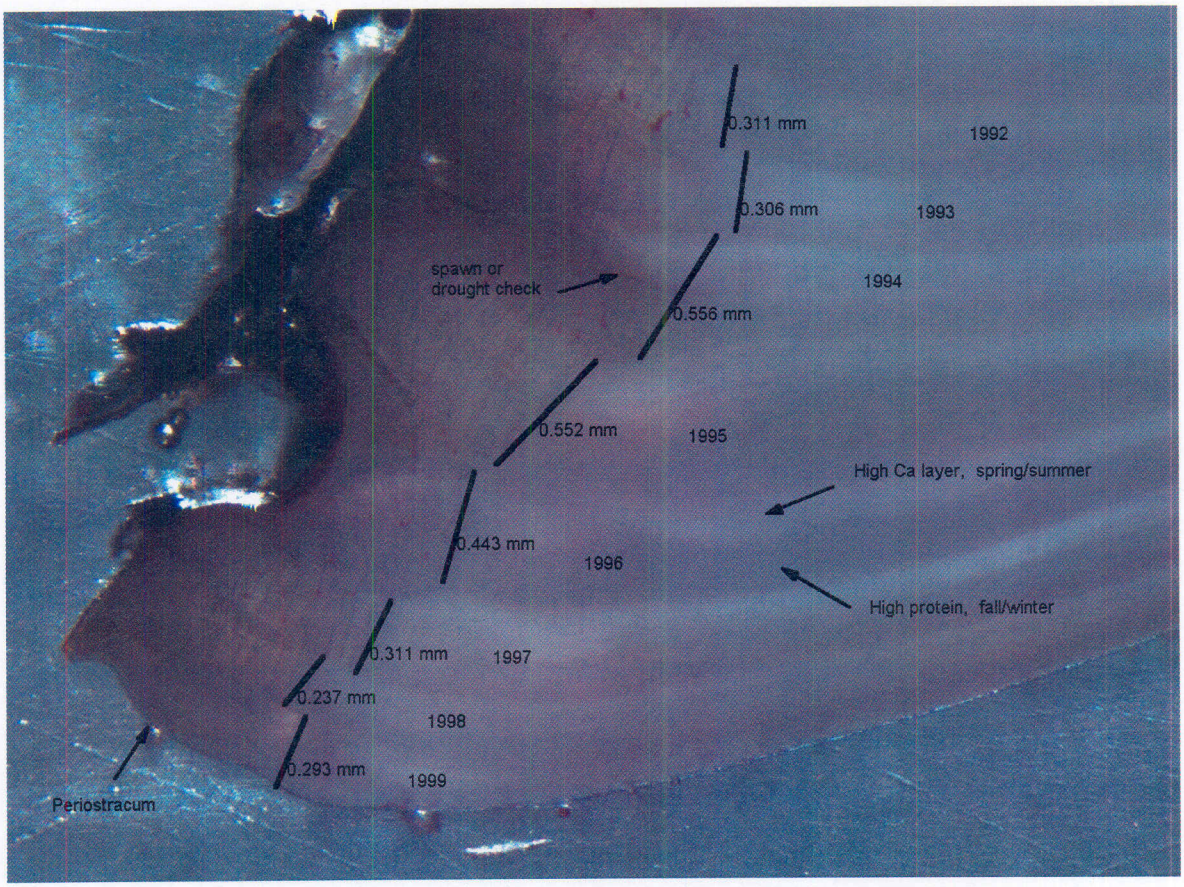
Age estimations based on field measurements of length, and not on shell sections, should be used with caution, as such calculations may not reflect the actual age of a particular animal. Growth rates of individual unionids, as with all bivalves, can vary substantially, even among siblings living side-by-side. Age calculations provide an average of the estimated age of the animals of that size group. Actual age estimates must rely on shell sections and must consider local conditions. Age estimates rely on annuli formation, which depending on temperature, food supply, and other still unknown conditions, may not be regularly deposited. For the purposes of this study, considering winter temperatures and examination of the shell matrix, we have assumed that internal annuli, consisting of dark proteinaceous bands alternating with light highly mineralized bands are being formed on an annual basis, with the proteinaceous bands indicating the winter season (Figure 25). Spawning checks and other disruptions or cuts through the matrix do occur, but patterns of band periodicity and disruption of the shell matrix are visually quite different. We have also assumed that shell readsorption (reduction in shell length) is minimal, even in old animals.

Differences in growth rates and ages within each lake and between lakes, as represented by the regressions in Figures 23 and 24 were tested using ANCOVA. *Pyganodon spp.* grew significantly faster in Chickenbone Lake than in any other water body sampled ($n= 50$; $p=0.017$). Otherwise, there were no detectable significant differences ($p\leq 0.05$) in growth rates and ages of either *Pyganodon spp.* or *Lampsilis spp.* between most lakes analyzed to date (except lakes Desor and Siskiwit). The relationship between length and age in Desor *Pyganodon spp* is not significant – length does not necessarily reflect age. Other factors such as water temperature or food supplies exert greater influence on shell length than age of animal.

We used the estimates of age, and the basic length frequency data, to determine year class distribution, recruitment patterns, and the percent of the population that had not yet reached sexual maturity. Age of sexual maturity is easily determined in the Lampsilis spp., as the directional planes of shell growth can be seen to alter once reproduction

begins. This occurs at about year 5. Age of sexual maturity is less easily determined in *Pyganodon spp.*, but is also believed to occur between 4-5 years of age.

Figure 26. Representative labeled shell section photo is of an *Elliptio dilatata* from Grand Sable Lake, Pictured Rocks National Lakeshore, collected in 1999. This section shows yearly shell deposition, appearance of a drought check in growth, and spring/versus fall shell layers.



Chemical Contaminants

Only trace amounts of organic contaminants such as p,pDDE and a few PCB congeners were found in tissues of the clams tested, from any lake (Appendix 3). Though detectable, the levels found are well below any concentrations of concern. Location or species differences were not detected, but sample size was low.

The heavy metal concentrations in the tissues were again very low, even in lakes such as Sargent Lake where mercury concentrations in fish are problematic (Table 11). Siskiwit Lake usually had the highest metal concentrations of all the lakes tested, but usually in amounts less than the low probable effects concentrations, but were above threshold effect concentrations.

Quality assurance for metals data included analyses of duplicate clam samples, matrix clam spikes on separate samples, and reference material from the National Institute of Standards SRM 1566b (Oyster Tissue). The average recovery was 94.4% from spiked samples and 93% of reference material.

Other Invertebrates

In addition to the dense unionid populations several unique assemblages were noted in a number of lakes and are described here as a point of reference.

SPONGES- The most dramatic assemblage are the large, architecturally intricate sponge colonies found in lakes Chickenbone, LeSage, Livermore, Intermediate, and part of Lake Richie near the Intermediate Lake portage. While thin layers of sponges or small round colonies of sponges were found in many lakes, these were the only ones where tall sponges occurred. Taxonomic identification of sponges requires the use of resting stages or gemmules that form in the fall. Efforts are underway to obtain gemmules for further study.

ALGAL CLOUDS- The second unique assemblage was the plankton/algal clouds found only along the shoreline in lakes LeSage and Livermore. These greenish-colored, loose

aggregates consisted of multiple species of algae, bryophytes, zooplankton, bacteria, fungus, etc., and were often 4 meters long by 2 meters high.

LARGE SNAILS- The third invertebrate of interest was the large snails, *Bulimnea megasoma* (Haldenman 1841) reported to date only in Lake Siskiwit and in the Lake Superior shore just below the Malone Bay campground. The snails covered the rocks along the shoreline in waters at least 1.5 m deep.

Table 11. Heavy metal concentrations found in unionid soft tissue in Isle Royale waters, July-August, 2000. TEC= Threshold effects concentration. PEC= Probable effect concentration. Units=mg/kg.

	Barium	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Zinc
TEC		*0.592*	*56*	*28*	*34.2*	*0.2*	*39.6*	*159*
PEC '01 CONSENSUS		*4.98*	*111*	*149*	*128*	*1.06*	*48.6*	*459*
Chickenbone Lake	235	1.088	2.00	5.427	0.7561	0.0262	3.824	198
Intermediate Lake	239	0.773	1.27	2.461	0.960	0.160	0.325	104
Livermore Lake	92.6	0.2201	0.901	2.131	2.179	0.0152	0.347	54.1
LeSage Lake	113	0.838	1.86	3.70	1.124	0.016	2.405	115
McCargoe Cove	229	2.836	2.17	10.09	56.42	0.0147	0.741	78.0
Richie Lake	175	1.45	1.61	1.589	1.793	0.221	3.413	113
Sargent Lake	69.1	0.312	0.817	0.995	0.211	0.0051	0.139	49.7
Siskiwit Lake	502	3.905	7.72	14.1	1.622	0.0175	1.505	141
Whittlesey Lake	106	0.269	1.07	0.867	0.159	0.0206	0.296	44.5
Wood Lake	89.2	1.455	0.830	2.979	0.7123	0.0066	0.877	59.2

DISCUSSION

Unionid populations in these inland lakes of ISRO may be limited in species diversity, but high abundance and almost yearly recruitment patterns epitomize population dynamics as seen in the early 1900's (e.g. Baker 1928) but rarely seen anywhere in the Midwest at this time. These healthy and stable clam communities are an extremely valuable and an increasingly rare resource. We consider these populations to be healthy and stable based on the following characteristics: large population densities both in number/m^2 and estimated abundance; multiple number of year classes (from 2-65 years) indicating steady successful recruitment and long-term adult survival; and the presence of gravid females of both genera in all areas sampled indicating ongoing reproductive activity.

The density (#/m^2) of unionids found in ISRO ranges up to 33/m^2 which is comparable to densities reported elsewhere for mixed species communities and considerably higher than the 14/m^2 Hanson et al. (1988) reported for a *Pyganodon (Anadonta) grandis*-dominated lake in Alberta Canada. In our surveys of other national park unionid populations, lake densities have varied considerably, but the ISRO densities are the highest found to date. For example, the lakes at PIRO and ISRO share similar habitat characteristics and two of the same clam genera, *Lampsilis spp.* and *Pyganodon spp.* Yet, while the maximum density of clams seen in Isle Royale lakes was 33/m^2, the maximum density found in PIRO lakes of similar size was only 4.1/m^2 and estimates of population abundance were significantly lower (Table 12).

Table 12. Comparison of estimated clam population size and hectares of habitat available between lakes in Isle Royale National Park (ISRO) and Pictured Rocks National Lakeshore (PIRO), 2000. The estimated size of the population in Lake Chickenbone is based on *Pyganodon spp.* numbers only, so the total number of clams in this lake is underestimated. PIRO data found in Nichols et al. 2001.

LAKE	ESTIMATED TOTAL # CLAMS	HECTARES HABITAT
Big Beaver- PIRO	109,977	52
Little Beaver- PIRO	9,890	2
Chickenbone-ISRO	6,388,200	93
LeSage- ISRO	747,000	45
Livermore- ISRO	732,000	30

The high abundance of unionids in some of the ISRO lakes is another indicator of the health of these populations. Abundance estimates could only be obtained for four of the lakes sampled, due to lack of bathymetric or substrate-type data. Of these four lakes, the size of populations in Intermediate, LeSage, and Livermore is similar to that reported in the literature for unionid populations elsewhere, even though average #/m^2 were at times, lower at ISRO. For example, Hanson et al. (1988) estimated that Narrow Lake, which at 114 ha is nearly three times the size of LeSage or Livermore, contained 2,790,000 clams, with an average *Pyganodon spp.* density of 14/m^2. This is twice the average density of clams we found in Chickenbone Lake (7/m^2) but we estimate that Chickenbone supports a minimum of 6.4 million clams. The difference in this case relates to basin morphometrics. The unionids in Narrow Lake could not colonize much of the lake substrate since the lake was deep, with little littoral area, and much of the available area was below the thermocline. In Chickenbone, all areas of the lake were available for colonization by *Pyganodon spp.* The colonization of *Lampsilis spp.* on the other hand was limited by substrate preferences. Thus density alone is not a good predicator of overall population abundance. The abundance of clams in Chickenbone Lake, with an estimated 6.4 million *Pyganodon spp.*, is higher than any population estimate we have seen for lakes in North America. This high abundance estimate, which does not included *Lampsilis spp.* numbers, exemplifies the population development that can occur where all habitat needs are met in abundance. Unfortunately, our ability to accurately pinpoint these needs is limited.

Many factors such as food supplies, substrate stability, thermocline depth, basin morphometrics, and fish movement patterns will control the ultimate number of unionids present in a water body. For a number of reasons, the attempts we made to correlate specific environmental factors with unionid densities and abundance were not successful in this study. The first problem is that the environmental data provided by Kallemeyn (2000) is based on single data points rather than on a seasonal series of data points. Chlorophyll *a*, total nitrogen levels, etc., measure available food supplies but such values change rapidly across the season. Single data points cannot accurately assess the complex food resources supporting, or limiting, unionid populations. Secondly, there is strong likelihood that some key unionid habitat needs are not being measured, since all

habitat requirements for these animals are poorly understood. Other studies have commented on these difficulties. For example, Huebner et al. (1990), worked on a 27-ha Canadian Shield lake, dominated by *Anadonta* (*Pyganodon*) *grandis*. Maximum densities were 0.03/m^2, but the estimated abundance was only 36800 individuals, even though the authors considered the lake environment to be non-limiting for unionids. Thirdly, while minimal habitat requirements for some variables have been documented, population responses to variables above threshold levels has not been documented (e. g. Neck 1990). Certainly, the lakes at ISRO exceed the minimal environmental thresholds reported to be required by clams such as oxygen concentrations >3ppm and pH above 6.3 (e.g. Mackie and Flippance 1983; McMahon 1991;), calcium above 2-3 mg/L (Strayer et al. 1981), appropriate fish hosts such as yellow perch (e.g. Watters 1994), and suitable substrates above the thermocline (e.g. Miller et al. 1986; Neves and Widlak 1987). However, we cannot determine what factors are causing the variability in clam density and abundance between ISRO lakes with any degree of certainty at this time.

The greater question is why some water bodies contain no clams at all. No clams were found in any streams nor in three of the lakes sampled (Table 1). Unionid distribution in ISRO is somewhat atypical in that lakes, not streams, provide the only habitat available. In most other parts of the country, lotic systems are the prime habitat supporting unionid community development. Our hypothesis is that the streams in this park cannot support unionids because of the severe winter temperatures. These streams are shallow, have a bedrock base with only a thin covering of sand or gravel substrates, and thus provide no area for the unionids to burrow into during the winter to avoid low water temperatures and ice conditions.

The environmental conditions preventing colonization of clams in Feldtmann, Hatchet, and Leech lakes have proven more difficult to determine. As shown in Table 2 and 3, Hatchet and Feldtmann lakes lack clams, but have similar environmental conditions to lakes where clams thrive. The one consistent exception is the low phosphorus level (TP=7). Hatchet also has lower ammonia and nitrates than lakes containing clams and is very steep sided with little littoral zone. However, Feldtmann ammonia and nitrate levels are in line with lakes such as Livermore where clams thrive.

Similarly, important fish hosts such as yellow perch are very common in Feldtmann (Kallemeyn 2000). Koelz's survey in the 1940's did not find clams in Feldtmann either, which dismisses any hypothesis of recent extirpations. Our surveys indicate that the habitat in this lake appears to be excellent, with good substrate and lots of vegetation. Water depth permits fish to survive, so winter kills on clams would not be a problem. We do not know why clams are not in this lake. One hypothesis that needs further testing is that copper levels might exceed unionid threshold levels. Unionids as are all mollusks are very sensitive to copper concentrations and surface copper deposits do exist on ISRO. Neither Kallemeyn (2000) nor Whitman et al. (unpublished data) examined copper levels in fish or sediments from this lake. There is also the possibility that clams were never introduced into these lakes. In other parts of the country, historical changes in clam fauna, or population extinctions are traced through the use of old shell deposited in the sediment (middens). Unfortunately, due to the low calcium in the water, shell disintegrates rapidly once the unionid has died, and in some cases, while the unionid is still alive.

None of the other contaminants found in unionid tissues were at consensus deleterious levels (Table 11; Appendix 3). At this time, organic and metal contaminants are not directly affecting recruitment or long-term survival in unionid populations at ISRO, at least in lakes where clams occur. However, while the amount of contaminant involved per unionid may be very low, the massive amount of clam biomass in lakes such as Chickenbone is undoubtedly affecting contaminant transfer. If we calculate clam biomass using methodology as described in Hanson et al. (1988), then we estimate the 6.4 million *Pyganodon spp.* contain 62.4 metric tons (62400 kg) live weight, of shell and tissue. This would be an estimated 25,600 kg of dry biomass. If we then assume an even distribution of measured contaminants among this dry biomass, and calculate out the amount of contaminant per gram of dry biomass, then the clams in Chickenbone lake have sequestered 671 kg of mercury alone. Since predation on unionids appears to be low at this time, and long-term survival of individual clams is good, these bioaccumulated contaminants are effectively removed and sequestered from other lake biota for a number of decades. Changes in unionid abundance, recruitment, and

longevity will alter the amount of contaminants available to other biota in all these lakes. Further research is suggested to determine bioaccumulation patterns and turnover ratios.

The future of unionids on ISRO will depend on the degree of environmental changes that occur over the next century. However, the lack of correlation between environmental features and population size limits our ability to provide specific cause-and-effect relationships on how much change can be tolerated. Obviously with animals that survive over fifty years, temporal scale can be important. Though we prefer to stress the need to stabilize habitat conditions at currently levels as the best method of protecting unionids, we recognize that these populations have survived major environmental fluctuations during the past century.

Historical surveys are limited in number and scope but it appears that unionid distribution has changed little during the past century (Table 13). Unfortunately, species composition and density data are practically nonexistent. No species were recorded from the Koelz's 1940s survey and only one lake that we know contains clams, Lake Desor, was surveyed by Adams in 1906. Adams' 1906 survey is valuable in that it documented the presence *Pyganodon marginata*, which is comparable (synonymous) to the *P. cataracta* we recorded in 2001. Only Koelz (1940's) made any reference to abundance, and only in qualitative terms such as "scattered", "rare", "common". It was interesting to note that he said that clams were "rather scarce" in Lake Desor, and "abundant" in Chickenbone; both descriptors still hold true.

Using clam longevity data to track response to historical habitat change is also problematic as we have no background data for ISRO. The *Lampsilis spp.* at ISRO are not as old as we have found at PIRO, but are certainly older than found in other studies. At PIRO, a number of extremely old *Lampsilis luteola* and *radiata* were found. There, the oldest *Lampsilis spp.* was 145 years old and 90-year-old animals were common. In contrast at ISRO, the oldest *Lampsilis luteola* and *radiata* were about 63 years old. Thus, the oldest clam we found to date (estimated age- Table 10) was born just after the 1936 fire, before the wolves migrated over from the mainland and before the park was established in the 1940's. There is very limited age data published for other survey

locations, so we cannot determine whether the age difference between ISRO and PIRO *Lampsilis* is a normal population feature, or a die-off resulting from environmental changes, or even a reflection of good environmental conditions (anecdotal grow-fast-die-young unionid phenomenon). *Lampsilis radiata* is not always a long-lived animal. In one published study, the oldest *Lampsilis radiata* found in a Montreal lake were only 11 years old (Magnin and Stanczykowska 1971). The main point is that in spite of all known environmental changes over the last hundred years on ISRO, lakes containing clams 60 years ago, still contain clams today (Table 13) and 60-year-old animals do still survive.

We cannot determine if there have been any species extinctions during the past century. Unionid species composition found in ISRO is certainly limited, and disappointingly so considering the obviously prime habitat available on the island. The two genera found, *Lampsilis* and *Pyganodon*, are typical lake species dominating lake communities in the northern waters of North America. The lack of other genera, particularly *Elliptio* (spike) probably reflects a lack of introduction, not unsuitable environmental conditions. As mentioned earlier, historical clam faunas are usually determined by looking at buried shell. No such middens exist at ISRO, as calcium levels are so low that dead shell disintegrates rapidly.

While the *Lampsilis* and *Pyganodon* species found in ISRO are currently considered common, these genera are being rapidly extirpated throughout the Great Lakes drainage due to the range expansion of zebra mussels, suburban sprawl, and increased use of herbicides and pesticides. Zebra mussels present the most immediate threat. Unionid populations within the Great Lakes proper have suffered devastating losses from zebra mussel biofouling and food web changes, and have been almost completely destroyed in Lake Erie and Lake St. Clair. Severe population reductions are now occurring in lakes Michigan and Ontario. Zebra mussels have also expanded into inland waters, and are now found in 160 inland lakes in Michigan alone and in most of the lake-connected river systems in the lower peninsula. Unionid extirpation usually occurs within 2-4 years after such zebra mussel invasions. The "common" unionid species found in ISRO waters may within 10-15 years become one of the few populations

remaining in the state, if not in the entire Great Lakes watershed. We do not yet have data on the possible genetic uniqueness of these populations. However, recent studies on ISRO fish fauna suggest unique genetic strains of walleye and lake trout do occur in the inland lakes (Kallemeyn 2000). Further research on clam genetics will be needed to determine if any endemic species or subspecies do occur, and should focus particularly on the greenish-colored *P. cataracta* in lakes LeSage and Livermore.

A few of the lakes surveyed contained other unique invertebrate assemblages, specifically intricate sponge colonies, and algal clouds. These tall sponges were found only in 33% of the lakes sampled, and were concentrated along the Chickenbone/LeSage corridor (Table 1). Sponges are common organisms in most water bodies, but such tall sponges are extremely uncommon in freshwater. They have been documented in a few isolated lakes by Frost et al. (1982), Frost (1991), and from literature early in the 1900's (e.g. Neidhoefer 1940). These organisms can have substantial impact on the energetics of lake systems. Frost et al. (1982) found at one lake, that contained an average sponge biomass of 3.5 gm/m^2, the sponges filtered 10 million liters of water a day, and accounted for a major amount of the lake's primary productivity. In another study, Frost and Williamson (1980) documented that the amount of chlorophyll contained in the sponges was equivalent to the amount contained in their lake's entire phytoplankton community. Sponges are also filter feeders and are likely in direct competition with the unionid fauna for food and mineral resources. Their relationship with dense unionid fauna needs further research as does the environmental factors supporting these assemblages, as well as their impact on contaminant cycling in the lakes. The algal clouds have never been referred to in the literature and factors contributing to their formation are unknown.

Table 13. Historical clam distribution in lakes in Isle Royale National Park (ISRO).

LAKE	1999-2001	1908	1940's[1]
Ahmeek			present
Amygdaloid			present
Angleworm			present
Beaver			present
Benson			Not mentioned
Chickenbone	present		present
Desor[4]	present	present	present
Dustin			present
Epidote			present
Eva			Not mentioned
Feldtmann[4]	absent		absent
Forbes			present
George			present
Halloran			present
Harvey			present
Hatchet	absent		absent
Intermediate	present		present
John			present
"Leech"	absent		absent
Linklater			present
Livermore Lake	present		present
LeSage Lake	present		present
Mason			present
McCargoe Cove	present		present
McDonald			present
Mud			Not mentioned
Newt[3]			Not mentioned
Otter			present
Patterson			present
Pidote			present
Richie Lake	present		present
Sargent Lake[3]	present		present
Shesbeeb			Not mentioned
Sholts			Not mentioned
Siskiwit Lake[4]	present		present
Stickelback[3]			Not mentioned
Sumner			Not mentioned
Theresa			present
Wagejo			absent
Wallace			Not mentioned
Whittlesey	present		present
Wood	present		not determined

UNIONID POPULATIONS AND THREATS TO THEIR FUTURE STABILITY

There are a number of threats that may affect the future of the unionid populations in ISRO.

I. **Exotic species**. The main threat facing these unionids is the possibility that exotics species such as zebra mussels or round gobies will inadvertently be introduced into the lakes. Zebra mussels comprise the greatest threat, since once established in the lakes, their biofouling and food competition would result in rapid extirpation of the entire native clam fauna. The impact of round gobies would be subtler, through the displacement of the clam's native fish hosts. Another exotic species, the rusty crayfish, would have limited impact on unionids, but in high numbers, could remove all macrophytes in inland lakes, seriously impacting moose populations. Rusty crayfish and round gobies may also destroy the sponge fauna. Zebra mussels will have minimal impact on sponges.

Introduction of exotics into the inland lakes will likely be a two stage invasion, with preliminary invasion into Lake Superior waters surrounding the ISRO, followed by a secondary invasion into inland lakes. Due to the isolated nature of ISRO, human transport will be the main vector for the first stage of invasion, but not necessarily for the secondary move into inland waters.

Human transport of zebra mussels usually occurs through inadvertent movement of zebra mussels attached to motorboats, ferries, anchor lines, SCUBA gear or even research equipment such as gill nets or plankton nets. Zebra mussels can survive outside of water for weeks under cool, humid conditions. We consider the risk of introduction of this exotic to be high through the movement of motor boats from infected areas, such as Duluth harbor, to mooring areas around ISRO.

Once established, zebra mussels can move inland to some degree without further human assistance. Zebra mussels can crawl, though slowly, and are positively attracted towards running water. Risk of infestation is greatest for those inland

lakes which have gently sloping outlet streams draining into Lake Superior. For example, the stream leading from Chickenbone to McCargo Cove would be relatively easy for zebra mussels to colonize. Lakes whose outlet streams have steep gradients would be at lower risk for natural infestation.

Round gobies and rusty crayfish are different from zebra mussels in that the likely vector pathway is through use of live bait by fishermen. Once established in Lake Superior waters around the park, inland invasion again will be easy up outlet streams with low gradients and continuous flows. Isolated lakes, or lakes whose outlet streams follow steep gradients will be protected. The risk of inland migration by rusty crayfish is greater than that of round gobies, as crayfish are excellent climbers.

There are other exotic species currently in the news, such as the spiny water flea, *Bythyotrephes*. This zooplankter is in Lake Superior and has managed to invade an inland lake at PIRO. While likely that this animal could alter food web dynamics at ISRO, we consider its presence less harmful than other exotics, though it does indicate a vector pathway is present and active.

Of all the lakes sampled, we consider Chickenbone Lake to have the highest risk for the introduction of exotic species. This lake is unique among the lakes we sampled as it has an immense clam population in combination with large sponge communities.

Recommendations for Maintaining the Unionid Populations :

1. We recommend that use of live fish bait be banned from Lake Superior waters around the park unless the bait is purchased from pre-approved dealers who only sell farm-raised minnows or earthworms. The inland lakes at this time are restricted to artificial lures only, and this ban should remain in effect.
2. We recommend that NPS examine boats, canoes, and kayaks, shipped on the ferries during loading for the presence of attached zebra mussels or aquatic vegetation that might harbor zebra mussels.
3. We recommend annual late summer SCUBA diver surveys for zebra mussel colonies around public access sites in Lake Superior waters, at the ferry docking sites, underwater wrecks, motor boat docks, etc. Early infestations can be eliminated quickly by crushing the zebra mussels. Not all introductions become established populations!
4. We recommend monitoring the homeport of personal motor boats applying for docking permits. Most states maintain a list of zebra mussel/round goby infected ports, particularly through Minnesota Sea Grant and those boats should undergo cleaning or inspection before arrival.
5. We highly recommend that motorboats be excluded from docking in park waters, though we realize this option may be politically unfeasible. The plan to move motorboat dockage away from high-risk areas, such as McCargoe Cove with its access to low gradient outlet streams from inland lakes is excellent. In any event, such dockage areas need to be checked for zebra mussels by SCUBA divers at least once in late summer, unless records indicate a high number of boats visiting from zebra mussel infested ports-of-call when several interim inspections are suggested.
6. We recommend that natural barriers such as log jams, rock piles, etc., be added to, or at least not removed from outlet streams that have low gradients and easy access to Lake Superior. Such natural barriers may not hinder access by rusty crayfish, but will provide some protection from natural migration by zebra mussels or round gobies. Beaver ponds in particular could provide excellent

catchment basins where exotic species might shortstop and could easily be eliminated.

7. We recommend an increase in public awareness/education efforts to prevent the spread of zebra mussels, round gobies, and rusty crayfish. Programs on the ferries would be an excellent opportunity to program a captive audience. There is considerable amount of readily available educational material already prepared by Minnesota and Michigan Sea Grant programs. The new NPS Exotic Species coordinator, Linda Drees is familiar with this type of material and can provide further assistance.
8. We recommend that any SCUBA divers or snorkelers using inland lakes be questioned regarding previous contact with infested waters, and encouraged to wash all gear in hot water and dry everything between dives in Lake Superior and inland lakes.
9. We recommend that any research crews be strongly encouraged to follow decontamination procedures when moving between lakes. There are a number of decontamination protocols in existence, one of which we have, or contact Linda Drees for more information. Research crews at least need to be encouraged to work inland lakes first, and then Lake Superior waters.
10. We recommend the formulation of an exotic species containment/eradication plan before any problems occur.

II. **Fish Community Integrity.** Unionid recruitment and population densities appear to be better in lakes with high numbers of yellow perch and northern pike. Activities that might lower yellow perch numbers, such as stocking lake trout, overharvesting of yellow perch, etc, should be minimized.

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APPENDIX 1.

Detailed Study Plan Including Quality Assurance/Quality Control Project Plan (QAPP) For:

A SURVEY OF UNIONID MUSSELS IN THE AQUATIC SYSTEMS OF TWO NATIONAL PARK SERVICE UNITS: ISLE ROYALE NATIONAL PARK AND PICTURED ROCKS NATIONAL LAKESHORE

July 7, 1999

Prepared by: Approval Signature: Date:

Susan Jerrine Nichols
Section Leader, Benthic Ecology
U.S. Geological Survey
Great Lakes Science Center
1451 Green Rd Ann Arbor, MI 48105
Phone: 734-214-7218
Fax: 734-994-8780
E mail: S_Jerrine_Nichols@USGS.GOV

With assistance from other members of the small group QAPP planning group, including (see titles and addresses on distribution list, below):

Jack Oelfke, ISRO, NPS

Brian Kenner, PIRO, NPS

Roy Irwin, WRD, NPS

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Distribution List:

Susan Jerrine Nichols
Section Leader, Benthic Ecology
U.S. Geological Survey
Great Lakes Science Center
1451 Green Rd Ann Arbor, MI 48105
Phone: 734-214-7218
Fax: 734-994-8780
E mail: S_Jerrine_Nichols@USGS.GOV

Jack Oelfke
Isle Royale National Park (ISRO)
800 East Lakeshore Drive,
Houghton, MI 49931

Jeerry Belant,
Pictured Rocks National Lakeshore (PIRO)
P.O. Box 40,
Munising, MI 49862

Roy Irwin, Senior Contaminants Specialist and
Biomonitoring Coordinator
Water Resources Division, NPS
1201 Oakridge Drive,
Fort Collins, CO 80525

Project/Task Organization:

Key personnel and organizations that are involved in the project include:

Principal Investigator and Project Leader
Susan Jerrine Nichols, USGS, BRD

The principal investigator will be assisted by other BRD staff including, but not limited to: Michael Stewart, USGS, BRD, Indiana Dunes National Lakeshore (general project assistance); Mike Schloesser, USGS, BRD, Ann Arbor (general and malacological assistance); and Mike Hoff, USGS, BRD, Ann Arbor (statistical assistance)

Park Service Representatives involved in the project include:

Lead Contact/Project Coordinator for Isle Royale National Park
Jack Oelfke

Lead Contact/Project Coordinator for Pictured Rocks National Lakeshore
Brian Kenner

Technical Contact for the National Park Service Water Resources Division
Roy Irwin, NPS, WASO, Fort Collins, CO.

Data users will include the Park Service Staff, USGS staff, others doing bivalve studies in the region, and the general public.

Problem Definition and Questions to be answered:

The first six questions are qualitative and semi-quantitative questions. Questions 7-10 are quantitative questions related to establishing baseline condition status for later comparison with subsequent changes and possible determination of long-term trends:

1. What unionid and other easily identified species of bivalves are present in representative lakes and streams on ISRO and PIRO?

2. At all sites sampled, what is the abundance classification of each species (rare, common, or very abundant)?

3. At these same sites, which species fall into quickly ascertainable age classifications (i.e., juvenile, adult) based on size? Which species are actively recruiting?

4. What is the overall status of the population- stable, marginal, or at-risk?

5. With certain caveats, at these same sites, which of the unionid and other bivalve species fall into classifications such as native, non-native, pollution/disturbance tolerant or intolerant, rare, ecological sentinel species, or undesirable species?

6. What are the key environmental variables at each habitat sampled and are specific unionid communities associated with certain variables? Variables to be considered will be such things as which fish are and other aquatic organisms are present in the same area, type of substrate, dissolved oxygen, total calcium, pH, secchi depth, water depth, and water velocity,

7. What is the quantity of each species present based on randomized quadrats or transects?

8. What is the annual incremental increase in shell length, or growth rate, for each species (based on dead shell- may not be possible for all species or for any endangered species)?

9. What proportion of the population sampled is composed of individual unionids <5years of age?

10. What is the amount and type of chemical contaminant present per gm of soft body tissue for each species sampled.

Management. regulatory or additional study decisions or potential actions that might hinge on the results of the study include deciding:

1) If unionid and other bivalve populations in various Park lakes are in good shape, appear to be under stress, or are at risk based on current status.
2) What type of long term monitoring of unionids and other bivalves is needed (if any) to keep an eye on trends. In the final report, the Parks would like the principle investigator to make specific recommendations on the frequency of monitoring needed (in any), where/what to monitor, and specific monitoring protocols, etc. The recommendations should be very specific so that any Park Service natural resource manager in the future could understand what needed to be done to adequately document trends
3) Whether or not to try to eradicate or otherwise manage non-native bivalve species, hosts, or other biota that might be threatening native bivalve species.
4) What other management actions (if any) should be taken to see that unionids and other bivalves in ISRO and PIRO are protected according to NPS mandates.

Background Information and Previous Data:

The only background mollusk work available from ISRO was a report on the mollusks, mainly gastropods, found on the island (Walker, 1909). There is no existing unionid work available from the streams and lakes of PIRO. Probable mussel species that may be found at Isle Royale National Park and Pictured Rocks National Lakeshore are listed (Table 1, Dave Heath, WI DNR. A request to the Ohio State and Michigan State Mollusc Collections is ongoing to determine if unionids from these sites are present in their databases.

Previously collected data bases on environmental parameters (chlorophyll *a*, pH, secchi depth etc.) and fish communities for waters sampled will be examined and compiled for comparison with the unionid data collected by our survey.

Some initial "range-finding" and exploratory sampling will be done at PIRO to try the proposed methods and determine data variability (which can drive the number of samples needed). At this time, an effort will be made do fine tune optimum field methods and other study details. The QAPP may be modified based on the results of these exploratory efforts or the discovery of additional previous information or newly identified expert opinion.

Data Quality Objectives (DQOs):

General Introduction and Discussion of DQOs for Qualitative Questions (1-6):

The questions being asked are general ones. The information being collected is not being collected to respond to litigated issues or other issues expected to be especially contentious or otherwise be subject to any unusual scrutiny. The data is not being collected in response to Superfund (CERCLA) or Natural Resource Damage Assessment laws or other rigid processes that require particular protocols to be followed. So the guiding principal for DQOs in this project is simply scientific and general common sense (for example, does it pass the common sense and being able to say it with a straight face tests?) credibility. The questions being asked (see listing above) were divided into questions requiring qualitative versus quantitative answers to provide scientific credibility. For this modestly funded project, the QA/QC measures detailed in this plan should be adequate to insure that data collected will be of sufficient quality to answer the identified question(s) in a defensible manner. Precision, Accuracy, Representativeness, Completeness and Comparability (PARCC) terms are defined for qualitative and semi-quantitative questions as follows:

Precision: The variability of each set of repeat measurements will be quantified to give a simple indication of the precision (or lack thereof) of each method used. Precision is a measure of scatter among independent repeated observations of the same property. Using standardized protocols, optimal standard methods developed by an advisory team of experts, and trained teams, as specified herein, will all help minimize precision errors. In cases where many trial replicates are made, precision will be expressed as a standard deviation or relative standard deviation for normally distributed data or as some other measure of variability when the data is not normally distributed. In the case of the qualitative questions 1-6, reasonable quantitative DQOs are difficult to predict before the study is done. Also, the modest funding makes a high number of replicate trials impractical. Therefore, the professional judgement precision QC step taken for questions 1-6 will be that the principal investigator will present the results to at least one other malacologist and have that other person independently classify the results. The precision of the classifications made will be expressed as relative percent difference (RPD). The RPD is the larger value minus the smaller times 100 divided by the larger minus the smaller divided by two. The data quality objective is that the classifications will represent the best professional opinon of the principal investigator after getting an independent opinion of another malacologist and explaining the relative percent difference of opinions. The initial DQO for precision in the qualitative and semi-quantitative measurements is a relative percent difference (RPD) of 25% or less. In addition to this "professional judgement DQO", the following additional DQOs will be met to help insure adequate precision:

Precision will be estimated from repeated measurements. The investigators will ensure that 5% of the samples are resampled during the study by another team. In the case where use of a different team is impossible, such as dive samples in remote areas, the same team will repeat the sample immediately after the first sample is collected. Some of the samples will require cleaning and picking of young mussels from the sediment

collected. Each sample collected in this manner will be checked for completeness. Repeat samples will be handled the same as the original sample. The 5% of samples collected to check repeatability by the same team (or reproducibility among different teams) will meet a precision DQO of a relative standard deviation of 10% or less for repeatability (within team variation) and a precision DQO of 20% or less for reproducibility (between team variation).

Accuracy is a measure of confidence in a measurement. *Precision* and bias contribute random and systematic error in a measurement that together can negatively impact accuracy. Measurement accuracy can be determined by comparing a sample that has a known value, such as a standard reference material to the measurement result for that sample. Accuracy = average value minus the true value. For qualitative parameters such as secchi depth and macroinvertebrate abundance, however, no standard reference or performance evaluation exists. In these cases, the trainer's results will be considered the reference value and to which the trainees' results are compared. The DQO for accuracy in the qualitative and semi-quantitative measurements is a relative percent difference (RPD) of 25% or less.

Representativeness: The representativeness assessment is being done to insure that the data will be "representative" of the actual condition measured. Representativeness is defined as the degree to which the data represents a population parameter. This is affected by problems in any, or all, of the other attributes of data quality. Representativeness is also affected by the selection of sites to be sampled, the location of sites in a reach, and the time period when samples are collected. The random-stratified sample design is intended to maximize representativeness. The final study design will be reviewed by statisticians and study design experts to assure that the results are as representative as possible. The DQO for representativeness is to insure that the data is as representative as practicable by carefully following the randomization and other study design details (documented herein) that insure probability samples will be collected. If this is done, the data quality objectives for representativeness for the qualitative questions will be considered to have been 100% met.

Completeness: In a simple sense, completeness is a measure of the number of samples taken compared to the number originally judged to be needed to use the information. Valid data must be acquired from a minimum number of sites in order to make population estimates with a specified level of confidence. To calculate percent completeness (%C), we will divide the number off measurements that have been judged valid by the total number of measurements originally agreed upon as being needed and then multiply by 100. The DQO for completeness in the qualitative and semi-quantitative information is a percent completeness of 80%.

Comparability: Comparability is the extent to which data from one study can be directly compared to either past data from the current project or (better yet, and often absolutely necessary to examine trends or regional significance) to data from another study. It is difficult to interpret the meaning of data if the methods used are so unique that there is no comparison data available. Therefore, our "comparability" QC will insure that lab and field methods are similar enough to those used by other investigators to insure that data

will be "comparable" to high-quality data from other studies. The use of QA data, uniform training of field crews, and incorporation of team duplicate sample sites into the study, will all help insure comparability. Before study methods are finalized, an effort will be made to standardize our methods with those used in other studies in the state (the Michigan Mussel Committee), so that new data is comparable. The DQO for comparability in the qualitative questions is to insure that the data is as comparable as practicable by carefully following study design details documented herein. If this is done, and the data is therefore at least 95% compatible (RPD of 5% or less) with at least one other important data set in the region, the DQO for qualitative questions will be considered to have been 100% met.

Taxonomic accuracy is critical to all the questions being considered in this project. Standard operating procedures used to help insure taxonomic accuracy include the specification of the taxon level (species), the specification of appropriate taxonomic reference material, and voucher specimen collections. The DQOs for precision and accuracy in taxonomic identification are:
1) a relative percent difference of 5% or less between the identifications of the principal investigator and a museum taxonomic expert at the University of Michigan or other institution of equal or better reputation in the identification of bivalves, and 2) a relative percent differences of 10% or less between the identifications of the principal investigator and any others who help identify the bivalves in this project.

DQOs for Quantitative Questions (7-10):

DQOs for question 7 (What is the quantity of each species present based on randomized quadrats or transects.

Data collection for this question will involve destructive sampling, so precision and accuracy DQOs are difficult to develop. However, for this modestly funded project, the QA/QC measures for training, representativeness, comparability, and other PARCC parameters detailed elsewhere in this plan should be adequate to insure that data collected will be of sufficient quality to answer the identified question(s) in a defensible manner. During the initial stages of field sampling, the principle investigator will see if any practical quantitative DQOs for this type of data can be developed.

DQO for Question 8 (What is the annual incremental increase in shell length, or growth rate, for each species?):

The SOPs call for each shell section to be aged independently by two different people. The expert trainer will be considered to produce the correct value. The comparison results of the all the others doing this procedure (after training is complete) shall have a precision DQO of a relative standard deviation of 10% or less. Each trainee shall also have an accuracy DQO of a relative percent difference (RPD) of 10% or less compared to the results of the expert.

DQOs for Question 9 (What proportion of the population sampled is composed of individual unionids <5years of age.

The SOPs call for each shell section to be aged independently by two different people. The expert trainer will be considered to produce the correct value. The comparison results of the all the others doing this procedure (after training is complete) shall have a precision DQO of a relative standard deviation of 10% or less. Each trainee's results shall also have an accuracy DQO of a relative percent difference of 10% or less compared to the results of the expert.

DQOs for Question 10 (What is the amount and type of chemical contaminant present per gm of soft body tissue for each species sampled?):

Analysis techniques and QA/QC protocols to be used are described in Schmidt (1997), Schmidt and Hesselberg (1992), and Wilford et al. (1973). See Table 2 for detection limits.

QC samples used to help measure precision will include field and laboratory splits and duplicates. When more than two replicate measurements of the same sample are made, they are will be referred to as field (measuring both analytical and field precision) or lab (measuring precision of the lab analysis only) splits. As simple descriptive measures of variability, the relative standard deviation will be used to express the precision of repeated measurements of the same thing. When only two replicates are used, they will be referred to as duplicates and precision will be measured as the relative percent difference (RPD). The precision DQO for duplicate chemical analyses is 25% (or less) RPD. The precision DQO for spits chemical analyses is a 25% (or less) relative standard deviation. If the data seems to be from a non-normal distribution, quartiles will be used rather than 25% relative standard deviations.

Accuracy is a measure of confidence in a measurement. Measurement accuracy will be determined by comparing a sample that has a known value, such as a standard reference material to the measurement result for that sample. In the chemical analyses, QC samples will be used to help measure accuracy. The QC samples will include spikes (samples where the concentration of the chemical are known exactly. Percent recovery of the spiked material will be used to calculate analytical accuracy. The DQO for accuracy will be percent recovery of the laboratory control sample of 75-125%.

Representativeness: The representativeness assessment should insure that the data will be "representative" of the actual condition measured. Samples will be randomly selected to insure probability sampling. Precautions will instituted to make sure that samples neither add nor lose the contaminants being measured in transit from the point of collection to lab analysis, so that the concentration measured is actually representative of the concentration which was present in the field. QC chemical samples used to help measure representativeness will include

field blanks, equipment blanks, and rinsate blanks. The DQO for representativeness of chemical samples is a relative percent difference of 5% or less for each comparison of the sample blanks versus the controls.

To make sure the data is representative by avoiding false negatives, the following additional representativeness DQO will be used: 95% of all chemical analyses shall meet the following detection limits:

> Hexachlorobenzene, α- and γ-BHC, aldrin, dieldrin, endrin, α- and β-heptachlor epoxide, cis- and trans- nonachlors, p,p'-(DDE, DDD, and DDT), mirex (including 8-monohydro mirex), α- and γ-chlordanes, oxychlordane, toxaphenes (Cl 6 to Cl 10), and all other organochlorines not specified otherwise. Detection limits should be as low as state of the art permits and in no case higher than comparison benchmarks or higher than 0.01 ppm wet weight PQLs in tissues.
>
> Mercury: PQL detection limits 0.01 ppm (or lower) dry weight in tissues.
>
> Pentachlorobenzene, octachlorostryene, dacthal, and pentachlorophenyl methyl ether: Detection limits should be as low as state of the art permits and in no case higher than comparison benchmarks or higher than a PQL of 0.01 ppm wet weight in tissues.
>
> PCBs : Detection limits should be below the comparison benchmarks, by a factor of 10 whenever possible. Tissue detection limits in the ppb range are now possible (ATSDR. 1999. Toxicological Profile for Polychlorinated Biphyenyls). In no case should the PQL detection limits be above 0.05 ppm.

Completeness is defined as the percentage of measurements made that are judged to be valid according to specific validation criteria and entered into the data management system. Every effort will be made to avoid sample or data loss through accidents or inadvertence. The DQO for completeness in the chemical quantitative data is a percent completeness of 90%.

Comparability is addressed by utilizing standard EPA protocols from SW-846 guidance or the USGS Denver Water lab. When better methods are used, for example clean lab mercury methods with lower detection limits, only those methods which have already been used widely and gained scientific acceptance will be utilized. The (meta data) method details will be provided in the final report, along with a rationale explaining why the alternative methods are superior to standard SW-846 or Denver USGS water lab methods. The DQO for comparability for chemical data is that 95% must meet the criteria specified in this paragraph.

The initial DQOs specified above may be modified by the principal investigator with the approval of Park Service contacts if the results of the initial investigations at Pictured Rocks National Lakeshore indicate that modifications are necessary.

Implementation plan details. A summarization of project tasks and standard operating procedures (SOPs):

Approach and Methods

Although species richness in qualitative timed searches and in quantitative quadrat searches are correlated, more mussel species can be found in timed searches than in quadrat searches (Vaughn et al. 1997). Timed searches tend to overestimate obvious species and underestimate the less easily seen species. Quadrats will underestimate rare species and the total number of species, unless a very large number of samples are collected. A previous study required. 368 quadrats at a site to achieve a 95% confidence level (Vaughn et al., 1997). Therefore, we intend to use a combination of these methods as suggested by Vaughn et al. (1997). Finding the unionid beds in each river or lake and then concentrating quadrat sampling in these locations is a method that combines both qualitative and quantitative methodology.

SOPs for Site selection and Overall Study Design:

For qualitative sampling, the location of sampling sites chosen to survey within each habitat and park will be based on (1) A minimum of three sample sites (lentic, lotic and littoral zones) within each habitat type in each park, selected from literature and reconnaissance searches, and (2) a minimum of three sites within each habitat type will be surveyed by qualitative techniques. Qualitative sampling is faster and cheaper than quantitative and thus more sites will be covered.

For quantitative sampling, a minimum of three sites within each habitat type will be selected for quantitative sampling. This will be based on resource management recommendations and on both random and non-random lake stratification parameters.

Initially, sites will be chosen non-randomly to maximize our ability to locate unionid populations. The selection criteria to be used are as follows: first, waters known to contain unionids based on shell found in the area by either park personnel or other research teams. If a number of such sites are present, those waters connected to one of the Great Lakes or suspected of being infested with zebra mussels will be sampled first (sites at maximum risk). The second selective criteria will be to sample waters with previously collected information on habitat, fish communities, and water quality information.

However, since one of the goals of this unionid survey is to provide a data base that can be used to test developing national unionid-specific IBI and ICI strategies, we will overlay these non-random site selection criteria with a random site stratification and selection system. The selection system entails grouping lakes and streams into functional classes based on habitat characteristics obtained from previously collected data provided by the parks. These characteristics include habitat such as water depth, clarity, chlorophyll a, pH, temperature regimes, hydrology patterns, fish populations, etc. We will overlay the waters we have sampled with these groupings and ensure that representatives of each group have been sampled. We will then use principal component

analyses to compare populations/ habitat, or use a non-parametric statistics if unionid populations are minimal. This type of information should provide baseline information for predicting unionid communities in park waters that we were not able to sample, but for which habitat data is available.

The divers will be placed on a line across the stream or lake and will float as much of the water body as possible searching for unionids. Once unionid beds are located, a square meter grid will be set-up across the entire bed, if possible, or at least 100 square meters of the bed (chosen randomly if bed is larger than this). The divers searching for unionids within the grid will sample the entire grid on timed surveys (15 min/diver for a maximum of 30 min/100 sq. meter grid). Species type, shell length, sex (if shell dimorphic) gravidity, and any other characteristics will be noted for every animal found.

A further 10% of the grids will be excavated. A grid will be selected, then a ¼ m quadrat frame placed randomly in it, and the entire substrate down to a depth of 15 cm removed, sieved and replaced if possible in the quadrat. All unionids will be identified and any juveniles that cannot be identified will be photographed and returned to the substrate. Once the unionid beds in each water body have been sampled, an equal number of 100 square meter grids will be placed randomly in areas where no unionid beds are found, and sampled as described above.

If no concentrated unionid populations are found in the water body, then 10-10x10 m^2 will be randomly placed in the water body, across various depths, and 100% of each 10x10 m^2 grids will be examined as described above, and a further 10% excavated.

Once waterbodies are clustered into groups, we will randomly choose examples from each group, and compare and contrast unionid populations from each group. This system will be field tested at Pictured Rocks, where access to sampling sites is easier. This dual sampling regime will provide a model for estimating potential unionid communities in waters that cannot be sampled directly.

Initial sampling techniques focus on finding the unionid beds in each river or lake and then concentrating quadrat random sampling within these strata.

SOPs for sampling in large water bodies:

Random 'statistical' sampling techniques will be used in water bodies too large for a total and complete float by the SCUBA divers. We will use transect lines to cut across potential longitudinal aggregations of unionids. This method involves sending the diver on randomly selected compass headings from one side of the water body to the other, or from the center of the water body to one shoreline. Five transects per 90° on the compass rose will be chosen randomly. Quadrat locations along this transect line will be chosen randomly, but one within every ten meters. Each quadrat will be fully excavated to at least a depth of 15 cm and all substrate material sieved. All unionids will be handled as described above.

These two sampling methods will be compared and contrasted for sampling bias at one lake and one stream in PIRO, which is more easily accessible and if possible at ISRO. This should enable us to predict the probability of finding unionid populations using statistical sampling at both parks.

Sampling methods will be modified according to the habitat that is surveyed and will include both stratified random sampling techniques and statistical sampling techniques using SCUBA divers or snorkelers (when water depth is <1.5 m). The dive team manager retains the final authority to alter sites sampled when safety concerns arise.

SOPs for Training:

Training: Field crews will be trained in the methods to be used for collecting mussels by unionid experts from the GLSC. Field crews will consist of at least on person highly experienced at sampling unionid populations (GLSC crew) along with additional less experienced personnel.

SOPs for Taxonomic Accuracy

Prior to any fieldwork, the principal investigators will examine museum collections to become familiar with mussel fauna found in the region (see Table 1). The PI (Nichols) has a collection permit (# 99-1055) from the Michigan Department of Natural Resources for collecting unionids including endangered species. Appropriate personnel from the permitting branch will be notified regarding new sampling locations. Historic information on mussel communities within the parks and surrounding areas will be obtained through a search and review of the literature, examination of museum collections, and contact with regional malacologists.

Taxonomic accuracy will be evaluated by conducting independent identifications of voucher specimens by an experienced taxonomist. Species identification will be based on live shell and collected dead shell. In the field, the divers will collect any dead shell found and record where it was collected. This shell will be sent to the University of Michigan Mollusc Collection for verification of identification and be used to prepare a field guide for each site. We will take pictures and video of each type of live shell found in the field. Shell vouchers for each type of live shell found will be collected. Using voucher shells, the randomly collected dead shell from each site, plus pictures of live individuals and array shots at each clam bed, we can correct all field ID problems later. Taxonomic keys will be distributed to each team along with a photograph of each mussel that is expected in the area. Training will be provided by the GLSC team on how to set transects or grids, clear quadrats, do excavations, determine gravidity and measure environmental parameters. SOPs include the following:

Photographic records: All crews will carry a 35-mm camera, a digital camera, and if possible, an underwater video camera. A picture of habitat and specimens collected will be taken at the site. More than one mussel can be photographed per slide.

Specimen record: A representative of each mussel species will be collected from each site (excluding endangered species). These will be preserved and returned to the laboratory. Voucher specimens will be deposited with ISRO and PIRO managers, plus at the University of Michigan Mollusc Collection.

Collection confirmations by experts: The voucher collection will be sent to mussel experts at the University of Michigan Mollusc Collection, and/or any other expert preferred by Park managers for taxonomic confirmations.

All mussels collected (except for a voucher collection and animals needed for contaminant analysis) will be identified, photographed, and returned to the wild. Voucher specimens of each species will be retained and mussels of questionable identification will be kept for positive identification. All freshly dead shells collected will be stored in a bag containing a field label including stream or lake name, location, date, and collector. Voucher specimens will be narcotized and fixed by using menthol crystals until immobilized, then placed into 70% ethanol. A labeled reference collection will be made for each park for deposit with the park collection manager or the state museum. Pictures will be provided of any rare or endangered mussels for which no shell was collected.

SOPs for Quantitative Questions:

Question: What is the annual incremental increase in shell length, or growth rate, for each species (based on dead shell- may not be possible for all species or for any endangered species)?
Standard Operating Procedures to be used: The shell will be sectioned on a perpendicular line from the umbo to the ventral margin of the shell. The cut sections will be sanded using fine grade, coated in glycerin, and examined under a 10X power dissecting scope. Internal annular rings will be determined using techniques described in Tevesz and Carter (1980). Each shell section will be aged independently by two different people. Length and age frequencies will be plotted using a modified Walford plot (regression). Comparisons between internal and external annuli (examination for non-annular external rings) will be done according to the techniques described in Downing et al. (1992).

Question: What proportion of the population sampled is composed of individual unionids <5years of age?
SOP: The relationship between length and age will be determined through shell sections. Differences in age and length between sites will be determined as described above.

Question: What is the amount and type of chemical contaminant present per gm of soft body tissue for each species sampled?
SOP: Live individuals of two species of unionids, preferably P. grandis and L. radiata (if present), will be collected from two sites per park and placed on ice as quickly as possible and sent to the Great Lakes Science Center. There, soft tissues from each individual will be frozen at –40°F and processed individually.

The following contaminant array will be surveyed: pesticides including hexachlorobenzene, pentachlorobenzene, octachlorostryene, α- and γ-BHC, aldrin, dieldrin, endrin, α- and β-heptachlor epoxide, cis- and trans- nonachlors, p,p'-(DDE, DDD, and DDT), mirex (including 8-monohydro mirex), α- and γ-chlordanes, oxychlordane, toxaphenes (Cl 6 to Cl 10), dacthal, and pentachlorophenyl methyl ether; PCBs (80 congeners, including most of the planar dangerous ones) and mercury. Analysis techniques and QA/QC protocols are described in Schmidt (1997), Schmidt and Hesselberg (1992), and Wilford et al. (1973). Field and lab methods shall follow recommendations of EPA (SW-846) or published USGS protocol and shall be detailed as meta data in the revised QAPP submitted with the first annual report.

Question: With certain caveats, at these same sites, which of the unionid and other bivalve species fall into classifications such as native, non-native, pollution/disturbance tolerant or intolerant, rare, ecological sentinel species, or undesirable species?
SOP: The following caveats will be factored into to these designations: There are no non-native unionids presently found in the continental United States. There are no undesirable unionid species. While three species (Lampsilis radiata/siliquoidea, Leptodea fragilis, and Pyganodon grandis) are commonly found in all types of habitats, the term "undesirable" is probably inapprorpriate as it implies something that must be eradicated rather than just a very adaptable species. Although not unionids, zebra mussels, asian clams, and various fingernail clams will be documented and reported. Taxonomic identification of fingernail clams is difficult, but an attempt will be made to identify them to the lowest level practicable.

SOPs for Documentation of habitat. With each qualitative and quantitative sample, we will also collect habitat data. These will include composition of substrate, water depth, and presence or absence of zebra mussels. These include scoring for stream and lake habitat variables (see field forms in the appendix).

Schedule of activities

June 1999	Methods/Site selection discussion
July 1999	Reconnaissance/Sampling (PIRO)
August-early September 1999	Reconnaissance/Sampling at ISRO
Fall	Data entry
EOY	Report
June	Reconnaissance/Sampling at PIRO
August-early September 2000	Reconnaissance/Sampling at ISRO
Fall	Data entry
EOY	Report
June	Final report

Sampling dates will be scheduled after discussion with park managers and modified as needed. We have anticipated a total 28 days field sampling/park for the two-year period.

If sampling is completed sooner in one park, the remaining days will be spent at the other.

Statistics to be used:

General Approach:

We will use both general statistics (median, range, etc.) as well as multivariate statistical methods to analyze the abundance data (number of mussels/taxon/transect), comparisons between populations within a water body and water bodies and potential relationships to habitats.
In addition to the basic statististics described above, we will use multivariate statistical methods to analyze abundance data (number of mussels/taxon/transect/grid). Hierarchical cluster analysis (Afifi and Clark 1990) will be used to reveal groups and patterns in abundance data across habitats. Principal component analysis will be used to reduce the dimensionality of the data by obtaining linear transformations of the mussel taxa variables and to summarize the major sources of variation in the abundance data (Jackson 1991). Raw data will be provided along with statistically manipulated data.

Statistics Related to Specific Questions:

Question: What is the quantity of each species present based on randomized quadrats or transects?
Statistics to be used: Simple descriptive statistics will be provided for each quadrat/transect sampled and for each 100 sq. m plot sampled. We will provide the raw data on the actual number and species of unionids collected in each type of quadrat, the median and range for each specie, plus the calculated #/m^2. The type of statistics used to test differences between quadrats will be determined once we determine if the distribution patterns of these animals across the 100 sq m plot/transect are normal or skewed. If the distribution is normal, tests such as ANOVAs and standard deviations will be used to further characterize the population in this plot/transect. Non-normal distribution patterns will be initially analyzed using more descriptive statistics such as the average deviation from the mean (AVEDEV), median, quartile, quantiles, etc. If necessary the data will be transformed either using a log or arcsign transformation. Non-parametric statistics will be used only as a last resort. Different 100 sq m plots or transects within the same water body will be initially compared using the techniques described above, with the statistical tests dependent on the distribution of the data. We will use multivariate statistical methods to analyze the abundance data (number of mussels/taxon/transect/plot).

Question: What is the annual incremental increase in shell length, or growth rate, for each species (based on dead shell- may not be possible for all species or for any endangered species)?
Statistics to be used: A probability chart indicating the accuracy of estimating age through the use of external annuli (usable on live animals) will be prepared. Differences in growth rates for a single species within a 100 sq. m plot or transect as well as between different plots or transects will be determined using ANOVA or Tukey's t-test depending on the sample size.

Question: What proportion of the population sampled is composed of individual unionids <5years of age?
Statistics to be used: Length frequency histogram will be prepared for every species, every water body, and every 100 sq. m plot or transect.

Question: What is the amount and type of chemical contaminant present per gm of soft body tissue for each species sampled?
Statistics to be used: Simple nonparametric descriptive statistics (median, interquartile ranges, etc.) will be used to summarize the results.

Documentation and Records; Summarization of data handling QA/QC SOPs.

High quality, defensible data is required for all National Park Service projects, Data will be entered into an Excel spreadsheet and checked by the principal investigator. These data, at the completion of the project, will be transferred to the park for eventual entry into EPA's STORET database. Meta-data will be provided for all sampling protocols and data analyses. The following steps will be done to insure that data meets the quality necessary for the purposes of the project: All grid plots, unionid beds, etc., will be entered as meta-data into EPA Storet system. Locations of grids and unionid beds will be further delineated by GPS locations and maps provided to park managers.

Data handling QA/QC steps include making sure that: (1) transcription or data transfer efforts are minimized, (2) information is not lost, (3) chain-of-custody is followed where appropriate, and (4) appropriate decision makers get the results in a form they can understand. All water-related data, including physical, chemical, substrate type, and biological data, will be reported to the parks for eventual placement into EPA's newly expanded STORET database by national park service personnel.

Data will be entered into standardized forms with all blanks filled out, At each site, the site leader will check all forms for completeness. A photocopy of the sheet will be made prior to mailing. Data will be entered into Excel format and checked by the principal investigator. At the end of the project, the Excel database will be presented to the park.

The basics of guidance for data entry, data verification, data validation, data documentation, data archiving, data backup, and version control, will all follow the NPS I&M guidance (www.nature.nps.gov/im/dmproto/joe4OOOl.htm) as closely as possible within the practicalities of funding levels available. For example:

Data verification will include the verification of the accuracy of all entries by their comparison with the original source to identify and correct errors. This will include checking the accuracy of the computerized records against the original source.

Data validation will include reviewing field and computerized data for range and logic efforts (the pH can't be 25). Unlike data entry and data verification, data validation requires in-depth knowledge about the data. Corrections or deletions of logical or range efforts in a data set will be done with notations in the original paper field records about

how and why the data were changed. Modifications of the field data should be clear and concise but preserve the original data entries or notes (i.e., no erasing!).

Site identification by GPS. Site information will be recorded on a GPS unit or marked on a topographic map for later identification. These units, plus instruction on their use, will be made available to the field crews. Otherwise, the field crews will mark their sampling locations on topographic maps provided to them.

Data will be collected using the following data sheets (located at end of document):

Sheets 1&2. Stratified Random Field Sheet

Sheets 3&4. Statistical Sampling Sheet

Study Plan and QAPP Revisions

Provisions for the unexpected or alterations that need to be made in the final QAPP need to be anticipated. Unexpected situations often come up during the course of investigations and any major changes will need to be authorized by the Park representatives and WRD technical lead before being implemented. If changes are necessary, the QAPP will be revised accordingly as the study progresses.

The final QAPP will be attached (as an appendix) to the final report submitted to the Park Service. The QAPP plan thereby becomes an important part of post-project "meta-data" (data about data). The meta-data in the QAPP plan provides the detailed information reviewers need to understand exactly how the data was generated. Thus, the details of what was done must be available to those desiring to repeat the investigation exactly as it was done before. Access to these details is also critical to reviewers trying to understand data comparability, data representativeness, and other perspective on "what the data means." In peer-reviewed articles where attaching the entire QAPP is not allowed, an alternative way to include meta-data details of exactly what is done both in the field and the lab will be found.

Deliverables and other Reporting Requirements:

A. Interim Report - An interim progress report (EOY) win be due as an end-of-year report to the parks.

B. Final Report - Due EOY 2000 will be a draft final report to the parks. The final report is due June 2001 and will consist of the following parts:

1). Title page - listing the investigators and affiliations.

2). Abstract (suitable for an abstract journal).

3). Executive summary, management implications, and information needs.

4). Introduction

5). Methods (Brief)

6). Results

7). Discussion

8). Summary

9). Appendix I - species lists and abundance estimates per area sampled. Detailed maps of all areas sampled and where each species is to be found will be provided.

10). Appendix 2: Final Detailed Study Plan and QAPP including all SOPS, Detailed
Methods and metadata.

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Table 1. Unionid mussels that may be found in Isle Royale National Park and Pictured Rocks National Lakeshore (list compiled by David J. Heath, Wisconsin DNR).

Phylum Mollusca
Class Bivalvia
Order Unionoida
Family Unionidae

Subfamily Anodontinae

Anodonta cataracta cataracta (Eastern floater)
Anondonta cataracta marginata
Anodontoides ferussacianus (Cylindrical papershell)
Lasmigona complanata (White heelsplitter)
Lasmigona costata (fluted-shell)
Lasmigona compressa (Creek heelsplitter)
Pyganodon grandis f. grandis (Giant Floater)
Strophitus undulatus (Squawfoot)

Subfamily Ambleminae

Elliptio complanata (Eastern elliptio)

Subfamily Lampsilinae

Lampsilis cardium (=ventricosa) (Plain pocketbook)
Lampsilis siliquoidea (= radiata luteola) (Fatmucket)
Ligumia recta (Black sandshell)
Obovaria olivarioa (Hickorynut)

Table 2. PCB Congeners/trans nonachlor to be Determined by GC/NCI/SIM. Detection Limits for PCB Congeners and Trans Nonachlor for EPA Contract IAG DW14947842-01 (Remaining Pesticides to be Completed Before Analyses Begin)

Compound	Inst. Det. Lim. using 1 g sample (**ng/g** or parts/billion/gram dry tissue)
1. PCB Congener #31+#28	9
2. PCB Congener #33	4
3. PCB Congener #22	4
4. PCB Congener #52	12
5. PCB Congener #49	18
6. PCB Congener #47+#48	6
7. PCB Congener #44	25
8. PCB Congener #42	4
9. PCB Congener #41+#71	18
10. PCB Congener #64	4
11. PCB Congener #40	7
12. PCB Congener #63	0.4
13. PCB Congener #74	2
14 PCB Congener#70 + #76	1
15. PCB Congener #66	2
16. PCB Congener #95	6
17. PCB Congener #91	7
18. PCB Congener #56+#60	1
19. PCB Congener #84+#92+#89	1
20. PCB Congener #101	0.2
21. PCB Congener #99	0.4
22. Trans-nonachlor	0.08
23. PCB Congener #119	0.1
24. PCB Congener #83	0.6
25. PCB Congener #97	0.9
26. PCB Congener #81+#87	0.6
27. PCB Congener #85	0.3
28. PCB Congener #77	0.2
29. PCB Congener #110	0.5
30. PCB Congener #82	1
31. PCB Congener #151	0.02
32. PCB Congener #144+#135	0.03
33. PCB Congener #107	0.3
34. PCB Congener #123	0.1
35. PCB Congener #149	0.04
36. PCB Congener #118	0.3
37. PCB Congener #134	0.02
38. PCB Congener #114	0.4
39. PCB Congener #131	0.01

40. PCB Congener #146	0.01
41. PCB Congener #132+#153	0.02
42. PCB Congener #105	0.02
43. PCB Congener #141	0.1
44. PCB Congener #137+#176	0.08
45. PCB Congener #138+#163	0.04
46. PCB Congener #158	0.03
47. PCB Congener #129	0.01
48 . PCB Congener #126	0.03
49. PCB Congener #178	0.1
50. PCB Congener #175	0.1
51. PCB Congener #187+#182	0.08
52. PCB Congener #183	0.06
53. PCB Congener #128	0.02
54. PCB Congener #167	0.03
55. PCB Congener #185	0.04
56. PCB Congener #174	0.09
57. PCB Congener #177	0.1
58. PCB Congener #202	0.2
59. PCB Congener #171	0.1
60. PCB Congener #156	0.04
61. PCB Congener #173	0.06
62. PCB Congener #157	0.03
63. PCB Congener #200	0.2
64. PCB Congener #172	0.04
65. PCB Congener #197	0.04
66. PCB Congener #180	0.07
67. PCB Congener #193	0.08
68. PCB Congener #191	0.1
69. PCB Congener #199	0.2
70. PCB Congener #170+#190	0.09
71. PCB Congener #198	0.1
72. PCB Congener #201	0.3
73. PCB Congener #203+#196	0.4
74. PCB Congener #189	0.1
75. PCB Congener #195	0.1
76. PCB Congener #208	0.07
77. PCB Congener #207	0.1
78. PCB Congener #194	0.1
79. PCB Congener #205	0.2
80. PCB Congener #206	0.2
81. PCB Congener #209	0.07

82. Pentachlorobenzene	0.15
83. Hexachlorobenzene	0.6
84. Octachlorostyrene	0.5
85. p,p'-DDT	40.0

86. p,p'-DDE	10.0
87. p,p'-DDD	70.0
88. β-Heptachlor epoxide	2.0
89. Oxychlordane	1.0
90. Pentachlorophenyl methyl ether	0.5
91. Deildrin	0.5
92. Endrin	0.5
93. Aldrin	3.5
94. Lindane	1.0
95. Alpha BHC	4.0
96. Alpha Chlordane	0.2
97. γ-Chlordane	0.2
98. trans-Nonachlor	0.2
99. cis-Nonachlor	0.1
100. Tot. taxaphene	120.0
101. Dacthal	1.0
102. Photomirex	25.0
103. Mirex	2.0
104. Mercury	20.0

Internal QA/QC samples will include a blank, spike, duplicate, and reference unionid tissue samples (check) analyzed with each set of monitoring unionid samples. Additionally when each subject sample or QA sample is analyzed, internal standards of PCB congeners #136 and 204 will be added just prior to the analysis step to monitor sample injection and adjust instrument calibration for every sample analyzed. Also, each sample will be spiked with surrogates. The Great Lakes Science Center's current procedure requires that each sample is spiked (at least 50 times the measured background concentration) with PCB congeners #65 and 166 and octachloronaphthalene for the pesticides just prior to extracting contaminants from tissues. The relative response from these congeners is then compared to that obtained during the calibration step of the GC/MS and a recovery is then calculated. Results from the collected unionids are not usually corrected for recovery based on spiked samples. The purpose of the surrogate spike is to check each sample for different errors that may occur during sample preparation. Results from the surrogates are especially useful in determining extent of the problem and corrective action when spike or check results are outside the acceptance criteria.

Mercury content in unionid tissues is determined by using LECO High Frequency Induction Furnace. Reference samples are SPEC reference Plasma Standards. Blanks, replicates, and reference samples will be run with each set of unionid tissue samples.

A complete in-depth QA/QC and sampling handling protocol will be provided with the final report.

Susan Jerrine Nichols

Section Leader, Benthic Ecology **Phone: 734-214-7218**
U.S. Geological Survey **Fax: 734-994-8780** **Great Lakes Science Center** **Email:**
1451 Green Rd **S_Jerrine_Nichols@USGS.GOV**
Ann Arbor, MI 48105

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS
Michigan Unionid Committee, National Shellfish Association, National Unionid Conservation Society

PERTINENT GRANTS AND CONTRACTS
U.S. EPA: Reestablishing the Unionid Population in Metzger Marsh, Sept 1998- Sept 1999; $100,000.
U.S. National Biological Survey: 1) Development of refugia to protect Unionidae from zebra mussels and, 2) food web dynamics and nutritional requirements of Unionidae Aug. 1998- $100,000

MAJOR PUBLICATIONS:
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APPENDIX 2

Chemical Contaminant Data

I. Metals

	Barium (mg/kg)	Cadmium (mg/kg)	Chromium (mg/kg)	Copper (mg/kg)	Lead (mg/kg)	Mercury (mg/kg)	Nickel (mg/kg)	Zinc (mg/kg)
Chickenbone	235	1.088	2	5.427	0.7561	0.0262	3.824	198
Intermediate	239	0.7731	1.27	2.461	0.9599	0.016	0.3254	104
LeSage	113	0.8384	1.86	3.702	1.124	0.0156	2.405	115
Livermore	92.6	0.2201	0.901	2.131	2.179	0.0152	0.3473	54.1
McCargo	229	2.836	2.17	10.09	56.42	0.0147	0.7415	78
Richie	175	1.45	1.61	1.589	1.793	0.2206	3.413	113
Sargent	69.1	0.3123	0.817	0.9951	0.2109	0.0051	0.1389	49.7
Siskiwit	502	3.905	7.72	14.1	1.622	0.0175	1.505	141
Whittlesey	106	0.269	1.07	0.8671	0.1595	0.0206	0.2962	44.5
Wood	89.2	1.455	0.83	2.979	0.7123	<0.0066	0.8769	59.2

II. Organics

	PCB Congener Concentration (ng/g)								
	22	31+ 28	33	40	41+ 71	42	44	47+ 48	49
Chickenbone	0	0	0	0	0	0	0	0	0
Intermediate	0	0	0	0	0	0	0	0	0
LeSage	0	0	0	0	0	0	0	0	0
Livermore	0	0	0	0	0	0	0	0	0
McCargoe Cove	0	0	0	0	0	0	0	0	0
Richie	0	0	0	0	0	0	0	0	0
Sargent	0	0	0	0	0	0	0	0	0
Siskiwit	0	0	0	0	0	0	0	0	0
Whittlesey	0	0	0	0	0	0	0	0	0
Wood	0	0	0	0	0	0	0	0	0

	PCB Congener Concentration (ng/g)								
	52	56+ 60	63	64	66	70+ 76	74	77	81+ 87
Chickenbone	0	0	0	0	0	0	0	0	0
Intermediate	0	0	0	0	0	0	0	0	6.35
LeSage	0	0	0	0	0	0	0	0	13.65
Livermore	0	0	0	0	0	0	0	0	3.25
McCargoe Cove	0	0	0	0	0	0	0	0	12.55
Richie	0	0	0	0	0	0	0	0	0
Sargent	0	0	0	0	0	0	0	0	2.83
Siskiwit	0	0	0	0	0	0	0	0	4.24
Whittlesey	0	0	0	0	0	0	0	0	3.13
Wood	0	0	0	0	0	0	0	0	3.2

	PCB Congener Concentration (ng/g)								
	82	83	84+ 92+ 89	85	91	95	97	99	101
Chickenbone	0	0	0	2.6	0	0	0	0	0
Intermediate	0	0	0	2.68	0	0	0	0	0
LeSage	0	0	0	1.39	0	0	0	0	0
Livermore	0	1.92	0	2.86	0	0	0	0	0.68
McCargoe Cove	0	0	0	1.16	0	0	0	0	0
Richie	0	0	0	0	0	0	3.67	0	0
Sargent	0	0	0	0	0	0	0	0	0
Siskiwit	0	0	0	2.26	0	0	0	0	0
Whittlesey	0	0	0	3.25	0	0	0	0	0
Wood	0	0	0	2.09	0	0	3.11	0	0

	PCB Congener Concentration (ng/g)								
	105	107	110	114	118	119	123	126	128
Chickenbone	0	0	0	0	0	0	0	0	0
Intermediate	0	0	0	0.96	0	0	0	0	0
LeSage	0	0	1.46	0	0	0	0	0	0
Livermore	0	0	4.02	0.83	0	0	0	0	0
McCargoe Cove	0	0	3.68	0	0	0	0	0	0
Richie	0	0	2.42	0.81	0	0	0	0	0
Sargent	0	0	3.51	0	0	0	0	0	0
Siskiwit	0	0	3.49	0	0.41	0	0	0	0
Whittlesey	0	0	2.57	0.7	0	0	0	0	0
Wood	0	0	3.96	1.09	0	0	0	0	0

	PCB Congener Concentration (ng/g)								
	129	131	132+ 153	134	137+ 176	138+ 163	141	144+ 135	146
Chickenbone	0	0	0.25	0	0	0	0	0	0
Intermediate	0	0	0.3	0	0	0.55	0	0	0
LeSage	0	0	0.34	0	0	0.41	0	0	0
Livermore	0	0	0	0	0	0.25	0	0	0
McCargoe Cove	0	0	0.33	0	0	0.35	0.47	0	0
Richie	0	0	0.24	0	0.03	0	0	0	0
Sargent	0	0	0.43	0	0	0	0	0	0
Siskiwit	0	0	0	0	0	0.72	0	0	0
Whittlesey	0	0	0.34	0	0	0	0	0	0
Wood	0	0	0.34	0	0	0.42	0.96	0	0

	PCB Congener Concentration (ng/g)								
	149	151	156	157	158	167	170+ 190	171	172
Chickenbone	0	0	0	0	0	0	0	0	0.1
Intermediate	0.36	0	0.13	0	0	0	0	0	0.21
LeSage	0	0	0	0.11	0.22	0.22	0	0	0
Livermore	0	0	0	0.08	0	0	0	0	2.68
McCargoe Cove	0	0	0	0.08	0	0	0	0.13	2.54
Richie	0	0	0	0	0	0	0	0	2.14
Sargent	0	0	0	0	0	0	0	0	1.59
Siskiwit	0	0	0	0.19	0	0	0	0	0
Whittlesey	0	0	0	0	0	0	0	0	2.2
Wood	0	0	0	0	0	0.3	0	0	0.3

	PCB Congener Concentration (ng/g)								
	173	174	175	177	178	180	183	185	187+ 182
Chickenbone	0	0	0	0	0	0.13	0	0	0
Intermediate	0.13	0	0.26	0.25	0	0.16	0	0	0
LeSage	0.16	0	0	0	0	0	0	0	0.17
Livermore	0	0	0	0	0	0.26	0	0	0
McCargoe Cove	0.15	0	0	0	0	0.32	0.19	0.06	0.07
Richie	1	0	0.23	0	0	0	0	0	0
Sargent	0	0	0.66	0	0	0	0	0	0
Siskiwit	0	0	0.55	0	0	0	0	0	0
Whittlesey	0	0	0	0	0	0	0	0	0
Wood	0.2	0	0.41	0	0	0	0	0	0

	PCB Congener Concentration (ng/g)								
	189	191	193	194	195	197	198	199	200
Chickenbone	0	0	0	0	0	0	0	0.07	0
Intermediate	0	0	0	0	0	0	0	0	0.16
LeSage	0	0	0	0	0	0	0.1	0	0
Livermore	0	0	0	0	0	0	0	0	0
McCargoe Cove	0	0	0	0	0	0.25	0	0	0.06
Richie	0	0	0	0	0	0.12	0	0	0
Sargent	0	0	0	0	0	0.36	0	0	0.17
Siskiwit	0	0	0	0	0	0	0	0	0.18
Whittlesey	0	0	0	0	0	0.37	0	0	0.18
Wood	0	0	0	0	0	0	0	0	0.18

	PCB Congener Concentration (ng/g)								
	201	202	203+ 196	204	205	206	207	208	209
Chickenbone	0	0.13	0	0	0	0.28	0	0	0
Intermediate	0	0.2	0	0	0	0.66	0	0.05	0
LeSage	0	0.2	0	0	0	0.59	0	0.08	0
Livermore	0	0.26	0	0	0	0.84	0	0.09	0
McCargoe Cove	0.17	0.07	0	0	0	0.44	0	0	0.06
Richie	0	0.21	0	0	0	1.01	0	0.11	0
Sargent	0	0.26	0	0	0	0.7	0	0	0
Siskiwit	0	0.18	0	0	0	0.87	0	0	0
Whittlesey	0	0.21	0	0	0.78	0.23	0	0	0
Wood	0	0.19	0	0	0	0.63	0	0	0

	Heptachlor epoxide-B	Hexachlorobenzene	Mirex	OXYCHLORDANE	Octachlorostyrene	Aldrin
Chickenbone	0	0.293	0	0	0	0
Intermediate	0	0.448	0	0	0	0
LeSage	0	0	0	0	0	0
Livermore	0	0	0	0	0	0
McCargoe	0	0	0	0	0	0
Richie	0	0	0	0	0	0
Sargent	0	0	0	0	0	0
Siskiwit	0	0	0	0	0	0
Whittlesey	0	0	0	0	0	0
Wood	0	0.213	0	0	0	0

	trans-Nonachlor	Photo Mirex	p,p'-DDT	alpha-Chordane	cis-Nonochlor	Lindane
Chickenbone	0	0	0	0	0	0
Intermediate	0	0	0	0	0	0
LeSage	0	0	0	0	0	0
Livermore	0	0	0	0	0	0
McCargoe	0	0	0	0.18	0	0
Richie	0	0	0	0	0	0
Sargent	0	0	0	0	0	0
Siskiwit	0	0	0	0	0	0
Whittlesey	0	0	0	0	0	0
Wood	0	0	0	0	0	0

	Dieldrin	Pentachloro benzene	Endrin	p,p'-DDD	p,p'-DDE	Heptachlor epoxide-A
Chickenbone	0	0.226	0	0	0	0
Intermediate	0	0	0	0	0	0
LeSage	0	0	0	0	0	0
Livermore	0.313	0	0	0	0	0
McCargoe	0	0.201	0	0	0	0
Richie	0	0	0	0	0	0
Sargent	0	0.154	0	0	0	0
Siskiwit	0	0.176	0	0	0	0
Whittlesey	0	0	0	0	0	0
Wood	0	0.157	0	0	0	0

	alpha-Chlordane	Toxaphene	Toxaphene Cl10	Toxaphene Cl6	Toxaphene Cl7	Toxaphene Cl8	Toxaphene Cl9
Chickenbone	0	0	0	0	0	0	
Intermediate	0	0	0	0	0	0	0
LeSage	0	0	0	0	0	0	0
Livermore	0						
McCargoe	0.214	3.478	0	0	0.768	2.709	0
Richie	0	0	0	0	0	0	0
Sargent	0	0	0	0	0	0	0
Siskiwit	0	0	0	0	0	0	0
Whittlesey	0	4.165	0	4.165	0	0	0
Wood	0	0	0	0	0	0	0

APPENDIX 3 .

Background on the Unionid Genera and Species Found at ISRO

The distributions of *Lampsilis luteola*, the fat mucket, and *L. radiata*, are difficult to describe since no taxonomic authority can agree on whether these are true species, subspecies, or variants of *L. siliquoidea.* In general, this group of *Lampsilis* is widely distributed throughout the Mississippi drainage system (see Figure 3). The maximum size of these animals is about 13.5 cm, and the shell varies from extremely dark reddish brown, with no stripes, to pale tan with green stripes. Females have a posterior inflation to the shell (sexually dimorphic) and use a mantle lure to attract fish hosts. This mussel prefers quieter waters and has no limitations with regards to substrate. These are heavy shelled, slow-growing, long-lived animals. *Lampsilis* use a wide variety of fish as hosts for their larvae, including percids, centrarchids, and cyprinids.

Potamilus alatus, or the pink heelsplitter, is a large unionid, up to 15 cm in length, dark brown in color, with pink nacre. (In taxonomic keys from about ten years ago, this animal was called *Proptera alata*). Its presence in Grand Sable is unexpected. This mussel has not been reported from Lake Superior, although it is found in the Red and Winnipeg rivers in Canada. Our hypothesis is that these mussels were accidentally introduced into the lake. The only known fish host is the freshwater drum, which does not occur in Grand Sable, though glochidia-infected drum may have been accidentally stocked with some of the game fish.

Pyganodon grandis, or the giant floater, is the most adaptable widespread unionid in North America (see Figure 3). It is a fast growing, thin-shelled mussel, with no proven external sexual characteristics and can easily reach 26 cm in length. The shell is light-to-medium brown, usually without stripes, and inflated (roundish ventral edge). The nacre is white. This mussel is found in most habitats, except fast flowing areas, and at all temperature extremes. This mussel can use a wide variety of fish hosts.
Pyganodon cataracta cataracta, the lake floater, is more commonly found on the Atlantic slope. As with all *Pyganodon spp*, this is a fast growing, thin-shelled mussel, with no proven external sexual characteristics, but usually is less than 20 cm in length. The shell is elongated, medium-dark brown, usually without stripes and with white nacre. This

mussel is also found in most habitats, except fast flowing areas. This mussel can use a wide variety of fish hosts.

Unionids have a parasitic larval stage and most use a fish host to complete their development. The following is a list of the known fish hosts utilized by the unionid species found at Pictured Rocks. Source used Watters (1994)

Unionid Species	Known Fish Host
Lampsilis siliquoidea (both forms)	Black crappie, bluegill, common shiner, largemouth bass, pumpkinseed, rock bass, sauger, small mouth bass, walleye, white bass, white crappie, white sucker, and yellow perch.
Pyganodon grandis	Black crappie, bluegill, bullhead, carp, common shiner, darters, freshwater drum, gar, killifish, largemouth bass, pumpkinseed, rock bass, sauger, small mouth bass, stickleback, walleye, white bass, white crappie, white sucker, and yellow perch. In some localities, may not always require fish host to complete life cycle.
Pyganodon cataracta	Unknown, but assumed to be similar to *P. grandis*