



Status of Freshwater Unionid Populations at Indiana Dunes National Lakeshore.

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INTRODUCTION

Unionid mussels (freshwater clams) are the most endangered group of animals in North American waters (Williams et al. 1993). North America has the largest diversity of unionids in the world (Metcalf-Smith et al. 1998). 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, radionuclides, pesticides, human and feed lot wastes, mining wastes, acid runoff; and harvesting for shell and pearls (Turner and Rabalais 1994, 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 (Schloesser and Nalepa 1994, Strayer and Smith 1996). 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 within the Great Lakes Basin, including Indiana Dunes National Lakeshore.

Objectives:

1. What unionid and other easily identified species of bivalves are present in the lakes and streams of Indiana Dunes National Lakeshore?
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 and other aquatic organisms are present in the same area, type of substrate, dissolved oxygen, total calcium, pH, turbidity (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 <5 years of age?
10. What is the amount and type of chemical contaminant present per gm of soft body tissue for each species sampled? This will be a limited survey designed to locate impacted areas where further study would be warranted.

- 11.** Management, regulatory, or additional study decisions or potential actions that might hinge on the results of the study include deciding:
- a. Are unionid and other bivalve populations in various Indiana Dunes lakes and rivers in good shape, under stress, or at risk based on current status?
 - b. What type of long term monitoring of unionids and other bivalves is needed (if any) to keep an eye on trends?
 - c. Should we try to eradicate or otherwise manage non-native bivalve species, hosts, or other biota that might be threatening native bivalve species?

METHODS

The sampling program in Indiana Dunes National Lakeshore included initial visual scouting of rivers and lakes in order to determine where unionids are presently located (Appendix 1; Figures 1-8). Details of the sampling regime can be found in the attached QAPP (Appendix 2). Qualitative methods involved searches for at least one hour, using three people at each site. Searchers waded in the water and felt for shell in the substrate. The shoreline was visually searched for the presence of shell residue or middens as would be found in muskrat areas. Quantitative methods as discussed in the QAPP were not implemented since no live unionids were found.

GPS positions collected at each of the sample sites and shown in figures 1-8. Latitude/longitude points are provided for each site and indicated in the figure by a red asterisk. The yellow line indicates the area actually surveyed in the search for unionids.

RESULTS

A number of sites were qualitatively surveyed at INDU (Appendix 1; Figures 1-8). No live unionids were found in any waters of INDU. Dead shell was found at only one location in the Little Calumet River, station #8 (see Figure 6). Based on the condition of the shell and comments from local landowners, these unionids have been dead for over 30 years. The species found are listed in Table 1. We have identified the shell donated by Mr. Joe McCauley as follows:

Table 1. Species of unionid shell found in the Little Calumet River, Indiana Dunes National Lakeshore. Based on dead shell only.

Scientific Name	Common Name	Quantity
<i>Lampsilis ventricosa /ovata /cardium</i> group*	pocketbook	2 males / 1 female
<i>Lampsilis siliquoidea /radiata /luteola</i> group*	fat mucket	3 males / 1 female
<i>Amblema plicata</i>	three ridge	5
<i>Elliptio dilatata</i>	spike	3
<i>Lasmigona costata</i>	flutedshell	2
<i>Lasmigona complanata</i>	white heelsplitter	1
<i>Lasmigona compressa</i>	creek heelsplitter	1
<i>Pleurobema sintoxia</i>	round pigtoe	1
<i>Quadrula quadrula</i>	mapleleaf	1

* The name varies depending on which expert you talk to. I will officially refer to them as *L. ventricosa* for the first group, and *L. siliquoidea* for the second group. Unionid experts will know immediately which animal you are talking about.

This is a very typical population in the Great Lakes watershed. No species are considered endangered or threatened in Indiana. No dead shells were collected anywhere else in the park. Snails, sphaerids, and other mollusks were found at this site, but were not common. Snails and sphaerids were relatively uncommon in all our sampling areas, with the exception of the river in the state forest (see Figure 4, station #5). No zebra mussels or Asian clams, both exotic mollusks, were found in any INDU waters except Lake Michigan.

DISCUSSION

The lack of live unionid fauna at INDU is likely due to past habitat manipulation and ongoing pollution. We have not been able to find historical data from this site. There appears to be no prior reference collections of unionids from this area, based on contacts with the Chicago Field Museum, the Illinois Natural History Survey, and the University of Michigan Mollusk Collection. The presence of dead shell does indicate that at one time park waters were capable of sustaining unionid fauna. The lack of dead shell from other waters in the park might reflect a lack of historical presence, but may also be a result of the widespread streambed alterations occurring in this region over the past 100 years. Changes in bed location, water depth, and water velocity are very damaging to shell records.

The lack or low densities of other molluscs at most of our sampling sites is a potential indicator of ongoing water quality problems. The recovery of unionid fauna is often limited by factors of fish host presence, but such factors should not limit snail populations. Molluscan fauna is very sensitive to many contaminants, including heavy metals and various pesticides commonly used in agriculture.

There is of course the possibility that some small residual unionid population still exists somewhere in the park, or just outside its boundaries. A small, localized population would be easily overlooked by our sampling regime. We recommend that efforts be made by park staff to contact various user groups such as visiting scientists, fishermen, hikers, etc., and ask for any information on the location of dead shell and live animals.

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Appendix 1.

**Figures 1-8 showing sample sites for Indiana Dunes
National Lakeshore, 2002.**

Figure 1: Aerial photo mosaic map showing native unionid survey sites within the Indiana Dunes National Lakeshore (INDU), summer, 2002. Survey areas are highlighted in red. Detailed information is shown on Figures 2 - 8.

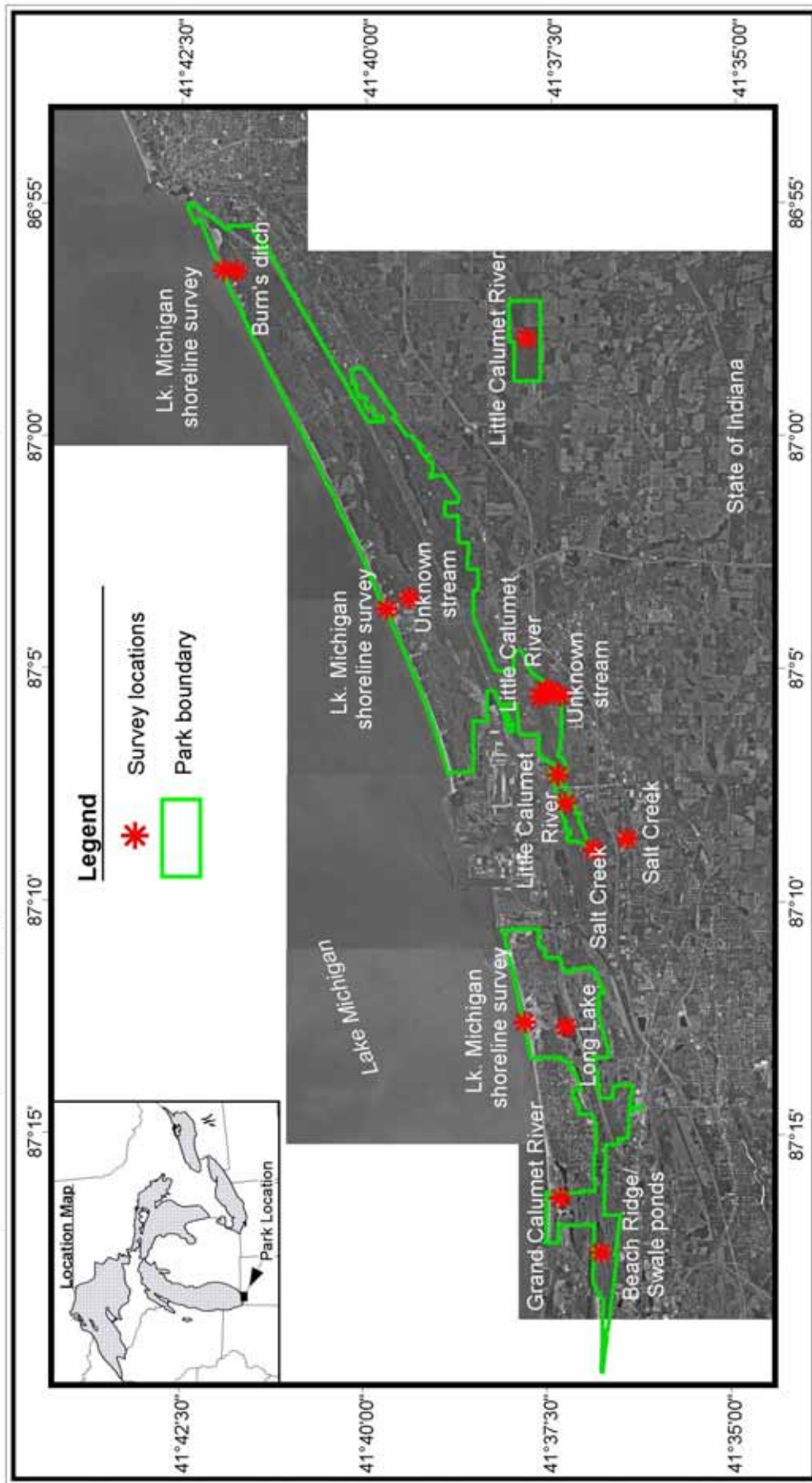
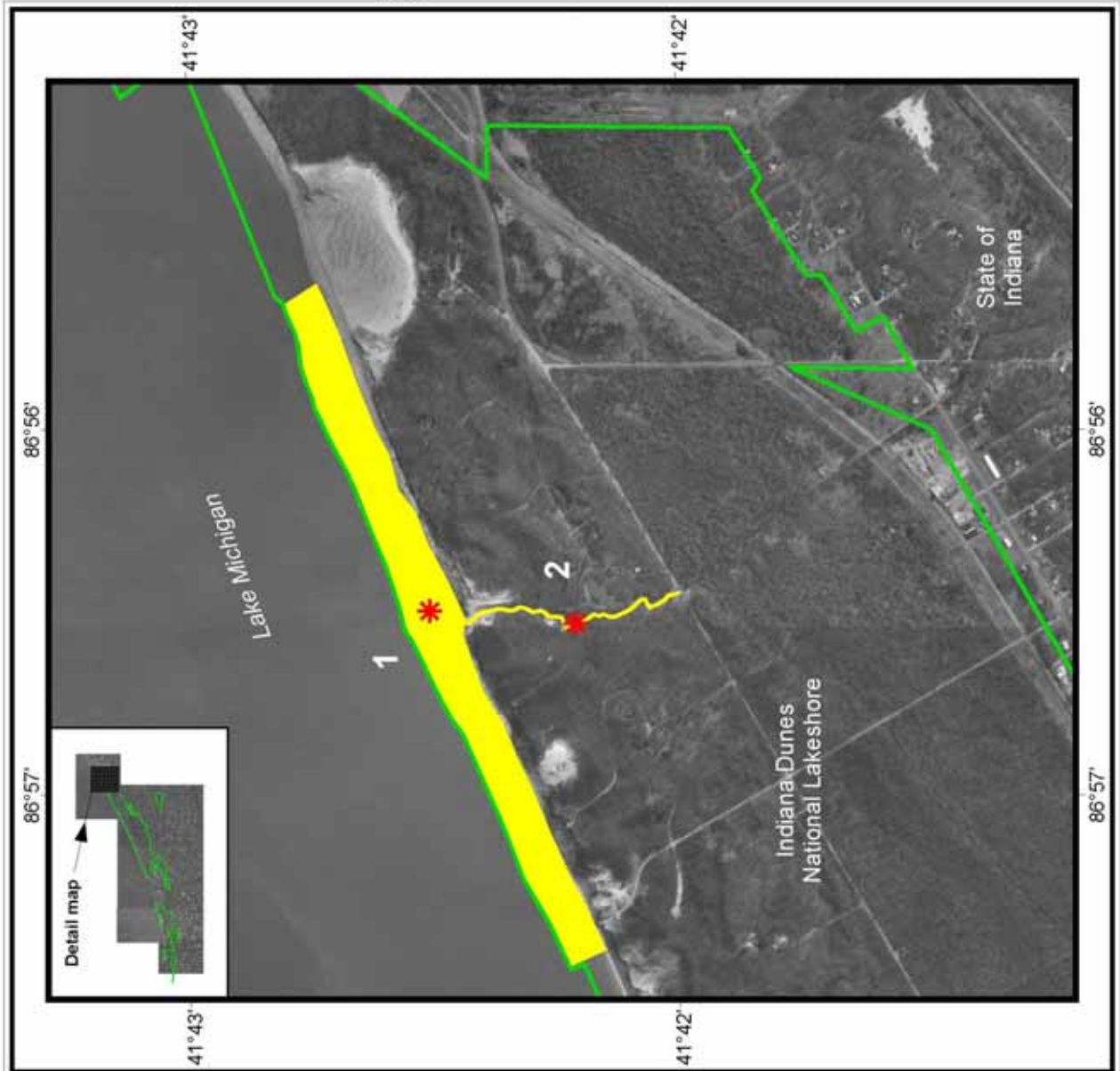


Figure 2: Detailed view of native clam survey sites on the northeastern portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.



Site description:

Site #:	Description:	Latitude:	Longitude:
1	Shoreline survey	41° 42' 26.8"	86° 56' 26.6"
2	Burns Ditch	41° 42' 13.8"	86° 56' 28.1"

Legend

-  Stream survey
-  Lake/pond survey
-  Survey site reference location
-  Park boundary



Figure 3: Detailed view of native clam survey sites on the southeastern portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.

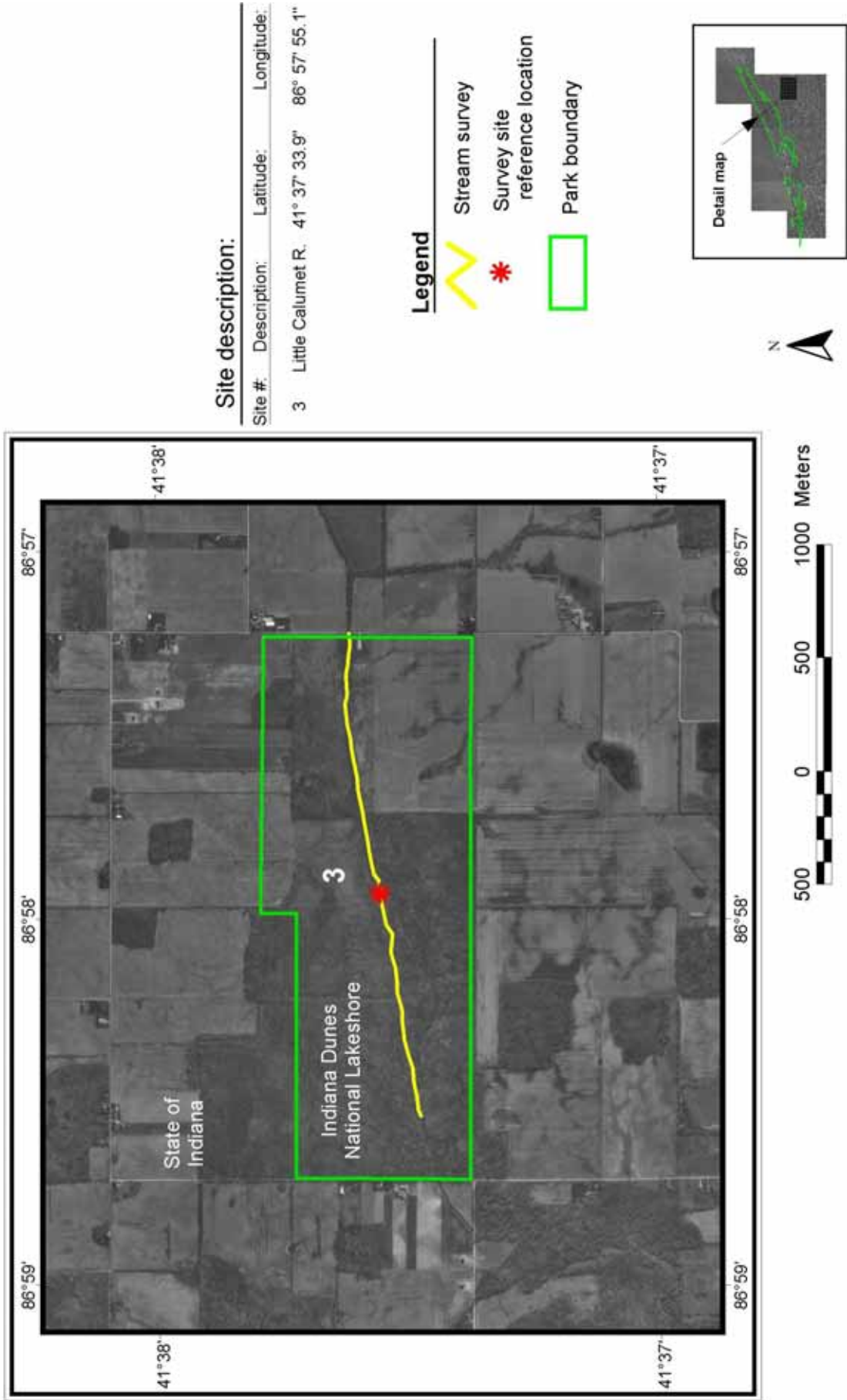
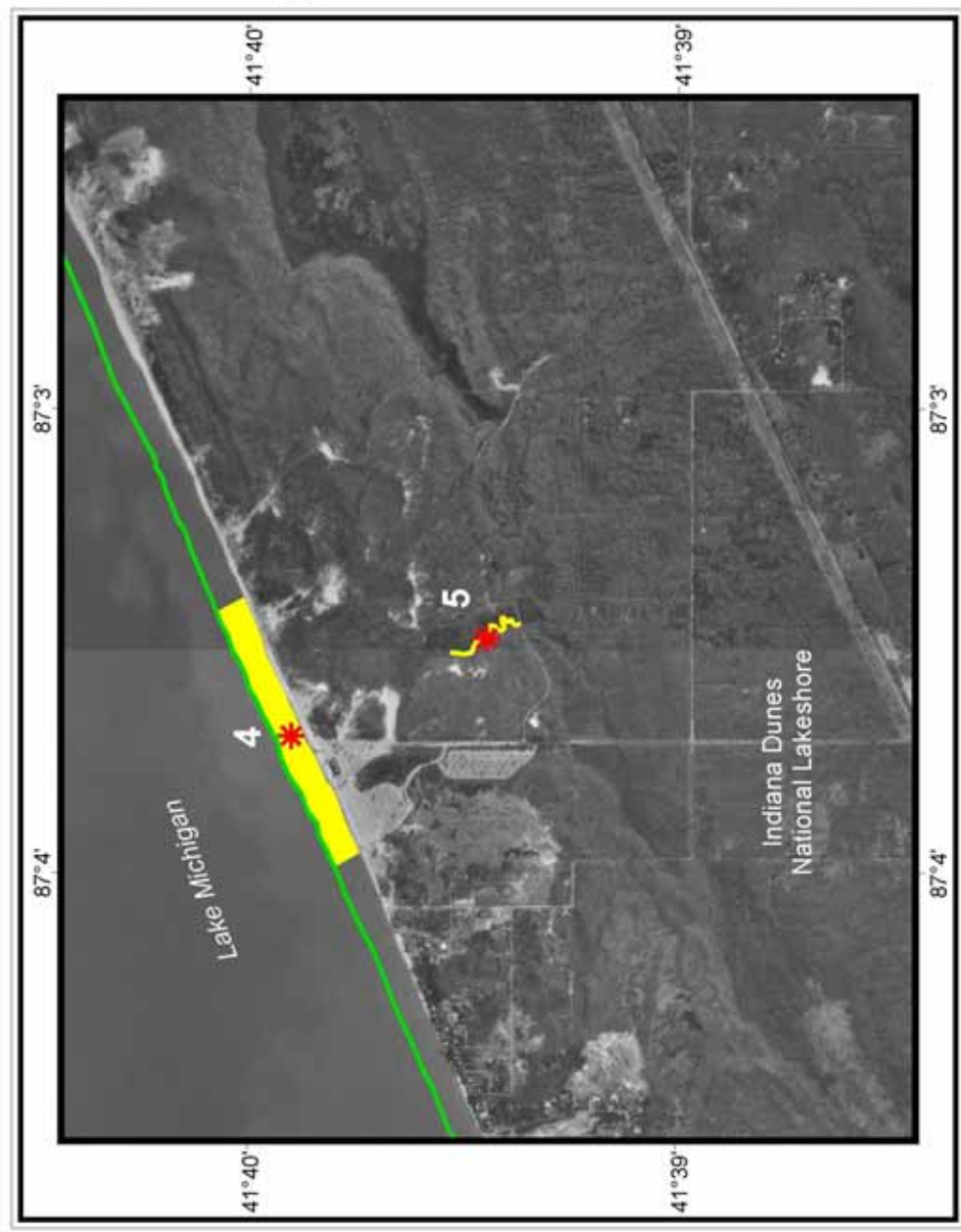



Figure 4: Detailed view of native clam survey sites on the north-central portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.



Site description:

Site #:	Description:	Latitude:	Longitude:
4	Shoreline survey	41° 39' 49.0"	87° 03' 44.3"
5	Unknown stream	41° 39' 27.4"	87° 03' 30.1"

Legend

-  Stream survey
-  Lake/pond survey
-  Survey site reference location
-  Park boundary

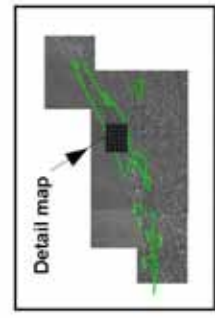
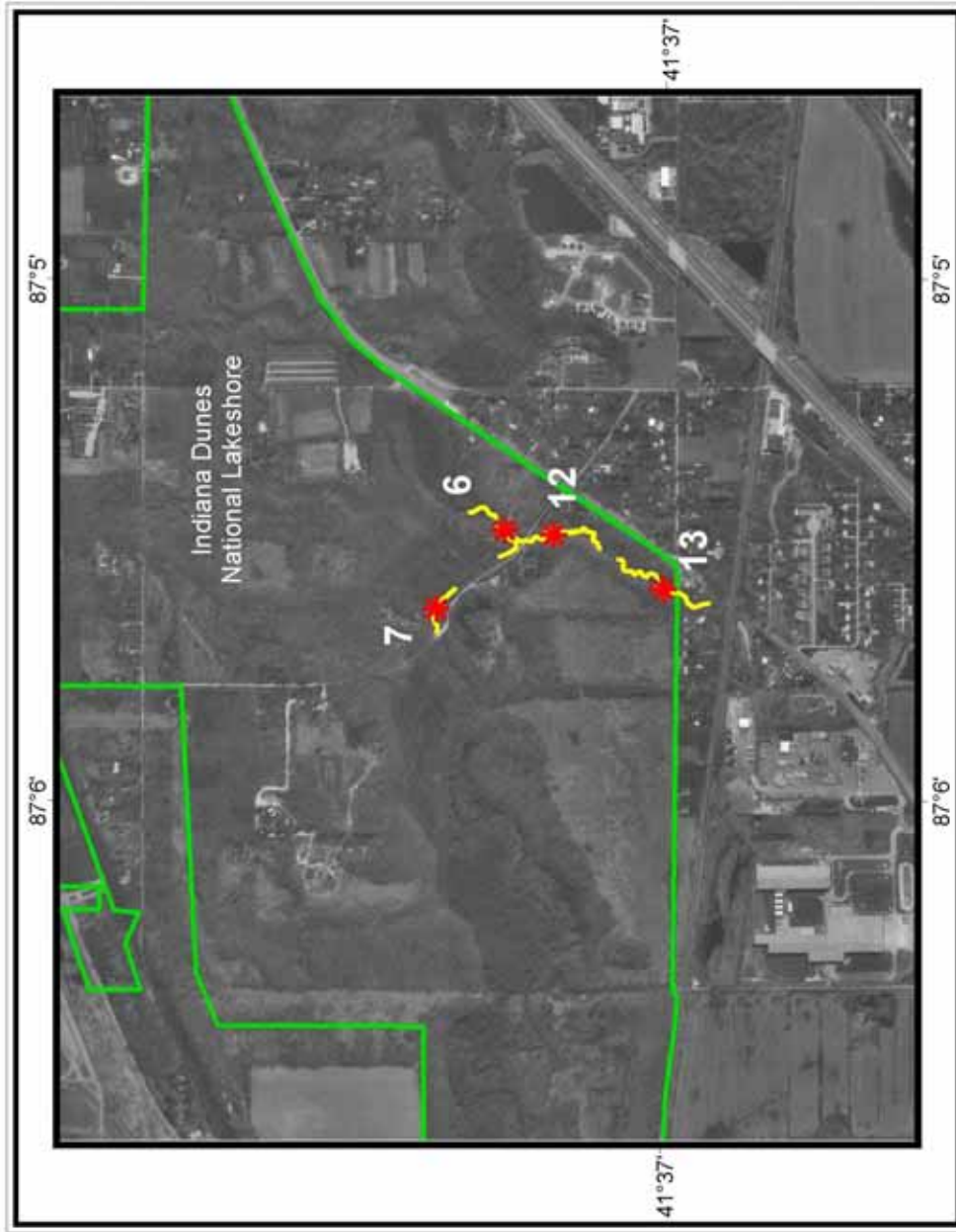





Figure 5: Detailed view of native clam survey sites on the south-central portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.



Site description:

Site #	Description	Latitude	Longitude
6	Little Calumet R.	41° 37' 15.1"	87° 05' 28.7"
7	Little Calumet R.	41° 37' 21.1"	87° 05' 37.9"
12	Unknown stream	41° 37' 10.9"	87° 05' 29.3"
13	Unknown stream	41° 37' 01.3"	87° 05' 35.6"

Legend

-  Stream survey
-  Survey site reference location
-  Park boundary

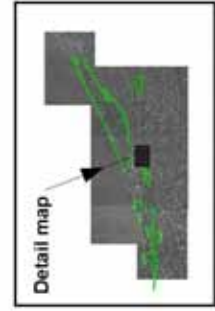
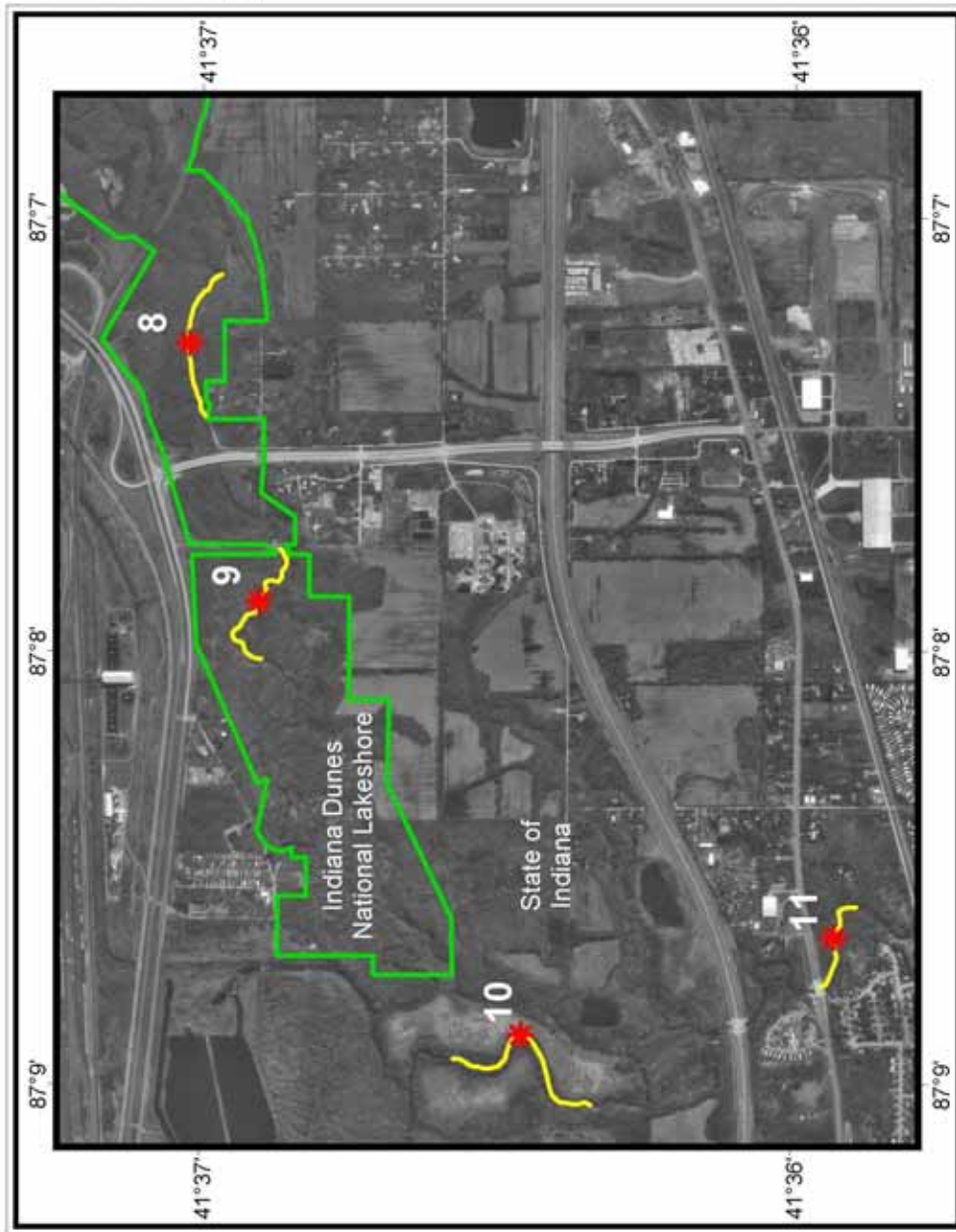





Figure 6: Detailed view of native clam survey sites on the south-central portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.



Site description:

Site #	Description	Latitude	Longitude
8	Little Calumet R.	41° 37' 03.7"	87° 07' 17.3"
9	Little Calumet R.	41° 36' 56.4"	87° 07' 53.0"
10	Salt Creek	41° 36' 29.2"	87° 08' 53.1"
11	Salt Creek	41° 35' 56.8"	87° 08' 39.6"

Legend

-  Stream survey
-  Survey site reference location
-  Park boundary

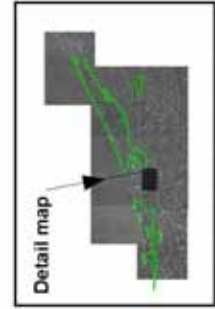
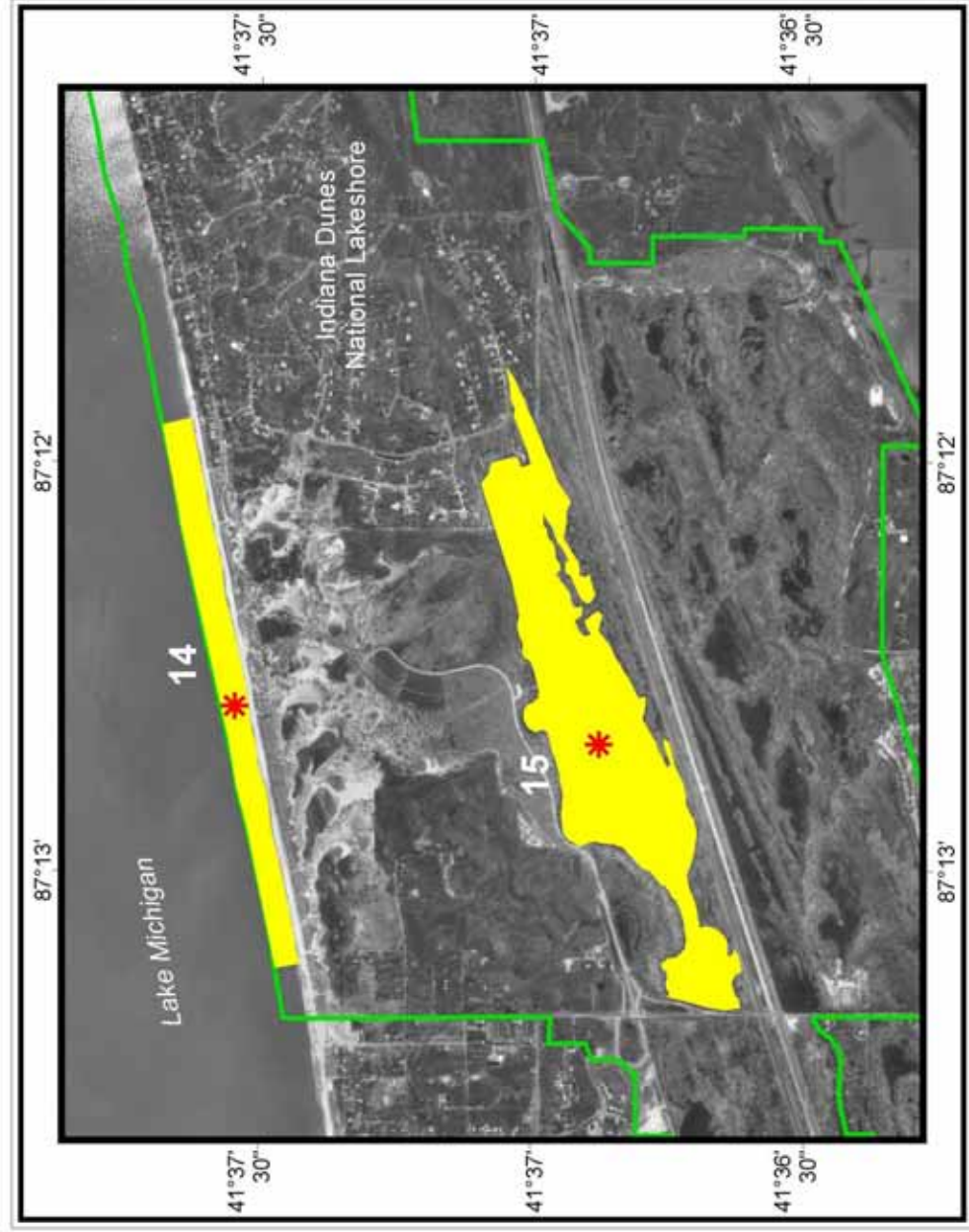


Figure 7: Detailed view of native clam survey sites on the northwestern portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.



Site description:

Site #.	Description:	Latitude:	Longitude:
14	Shoreline survey	41° 37' 35.3"	87° 12' 35.8"
15	Long Lake	41° 36' 55.2"	87° 12' 41.4"

Legend

- Lake/pond survey
- Survey site reference location
- Park boundary

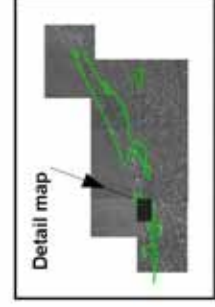
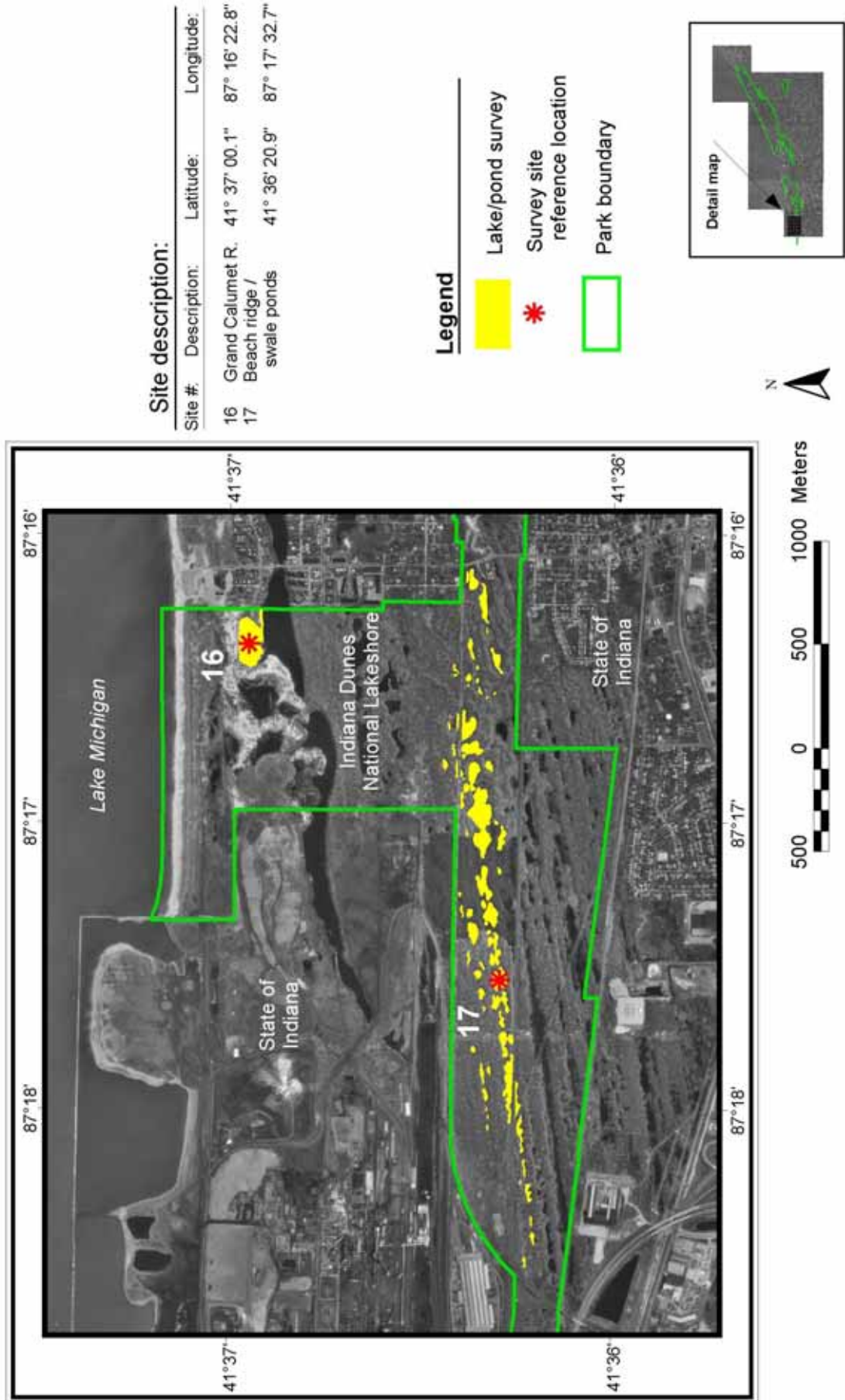


Figure 8: Detailed view of native clam survey sites on the western-most portion, Indiana Dunes National Lakeshore, summer, 2002. Survey location data listed in table below.



APPENDIX 2.**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**

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10. Statistics to be used
11. Documentation and Records; Data Handling QA/QC Standard Operating Procedures.
12. Reconciliation of Data Collected with Data Quality Objectives (DQOs).

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Project/Task Organization:

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

Principal Investigator and Project Leader

Dr. 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); Don 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:

- a. 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.
- b. 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
- c. 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.
- d. 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.

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 opinion 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 of 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 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 splits 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 be 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 rinse 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, oxychlordanes, 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, octachlorostyrene, 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 Biphenyls). 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 m² will be randomly placed in the water body, across various depths, and 100% of each 10x10 m² 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 one 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 gravity 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, octachlorostyrene, 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 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/silicoidea*, *Leptodea fragilis*, and *Pyganadon grandis*) are commonly found in all types of habitats, the term “undesirable” is probably inappropriate 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).

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 statistics 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/joe40001.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 errors 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):

